Ecology of Streams Contaminated by Acid Mine Drainage, near Reefton, South Island

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Iron flocs cover stream bed at Murray Creek.

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ABSTRACT

Physico-chemical conditions of surface and hyporheic receiving waters were investigated in relation to invertebrate community structure, and epilithic algal production, at ten stream sites near Reefton, South Island. Physico-chemical conditions, benthic and hyporheic faunas and epilithic algal production were sampled over a 12 month period from March 1998 to February 1999. Stream water pH ranged from 2.9 (strongly mine-affected sites) to 7.1 (control sites) with acid mine drainage-affected streams also having little or no measurable alkalinity and high conductivity, reflecting high concentrations of metal ions. Concentrations of total iron and total dissolved aluminium were elevated at low pH.

All streams with pH > about 4.5, had similar species richness and densities of invertebrates. In terms of numbers of species and individuals, the Plecoptera was the best represented insect order, followed by Trichoptera and Diptera at these sites. However, below pH 4.5, where concentrations of Fe were > 0.8 mg L^{-1} and Al > 0.4 mg L^{-1}, reductions in species richness and abundance were found and faunas were numerically dominated by Diptera, mainly Chironomidae. The presence of crustaceans, arthropods generally considered to be intolerant of acidic conditions, at nine of the ten sites, including those affected by acid mine drainage is noteworthy and consistent with the theory that many New Zealand streams invertebrates tolerate a wide range of physico-chemical conditions. In the laboratory, three species of insect tolerated higher concentrations of iron and lower pH than would be predicted from field surveys.

Epilithic algal biomass was lowest at the most acidic sites (pH < 4.5) where metal concentrations were elevated and precipitates prevented the attachment of algae to solid surfaces and may have inhibited photosynthesis. The greater species richness of streams with pH > 4.5 reflects the greater diversity of habitats and food resources available, in addition to their more equable water chemistry.

Lastly, the chemistry of hyporheic water samples was very similar to that of surface waters and furthermore, surface sediment and hyporheic faunas had much in common. However, the diversity of the hyporheos was lower, and Crustacea, followed by Diptera and Plecoptera dominated at all hyporheic sites. The notable and distinguishing feature of the hyporheic faunas was the strong representation of harpacticoid copepods.

The findings of this study indicate that not only are many New Zealand stream invertebrates found in both hyporheic and surface sediments, but at least on the West Coast of the South Island, they are also tolerant of low pH and elevated concentrations of iron and aluminium.

Chapter One

INTRODUCTION

The term "ecology" was first used by Ernst Haeckel in 1869. He defined ecology as "the total relationship of the animal to both its organic and inorganic environment" (Krebs, 1985). Ecology was later defined as "the scientific study of the interactions that determine the distribution and abundance of organisms" (Begon, Harper & Townsend, 1990). The distribution and abundance of aquatic organisms is determined by many interrelated factors. Winterbourn (1981) summarised these as large-scale factors including geology, altitude, geography, water chemistry and catchment type; medium-scale factors such as stream size, bed stability, gradient, hydrological regime, canopy, type of food available, and sources of nutrient enrichment and/or sources of contamination; and small-scale factors including substrate particle size, food substances, current velocity, and physico-chemical factors such as temperature and oxygen concentration. Some of these factors vary naturally over time, and the stream community is able to adjust to the normal range of conditions experienced. A change outside this range may not be tolerated by all of a streams inhabitants and the result may be disruption of "biological integrity" (Weber, 1981) characterised by organisms declining in numbers, migrating, or out-competing others (Penny, 1987).

In the Northern Hemisphere, the widespread acidification of many surface waters because of the occurrence of acid rain, has stimulated much interest in the effects of low pH on freshwater ecosystems and aquatic organisms (Collier & Winterbourn, 1987). Studies such as those by Haines (1981) and Giberson and Mackay (1991) have highlighted the deleterious effects of low pH and associated chemical changes on freshwater invertebrate and fish communities through reductions in species richness and density. The pH of most natural freshwaters lies within the range 4.0 to 10.0, but some extreme values have been recorded. Early in 1966 the Mount Ruapehu Crater Lake in New Zealand had a pH of only 0.9 (Bayly & Williams, 1973) on the other hand, a pH of 12.0 has been recorded in Lake Nakura in the African Rift Valley (Jenkins, 1932).

Acid precipitation is not a significant problem in New Zealand where the mean pH of rainfall is about 5.6 (Holden and Clarkson, 1986). Deposition of acid sulphur on the country is about 40 times less than in the worst affected areas of Europe (Winterbourn and Collier, 1987). Nevertheless, the West Coast of the South Island of New Zealand has many acid streams, both natural brown waters and others affected by acid mine drainage (Winterbourn and McDiffett, 1996). The acidity of brown water streams results from high concentrations of humic substances derived from decomposing organic matter in soil and swamps (Winterbourn and McDiffett, 1996).

Acid mine drainage is a major worldwide environmental problem that adversely affects both surface, ground (Gray, 1996), and hyporheic waters (Nelson *et al*, 1993). It is caused by the oxidation and hydrolysis of metal oxides (in particular pyrite) in water-permeable strata or in spoil dumped on the surface. This results in the formation of soluble hydrous iron sulphates, the production of acidity, and subsequently the leaching of metals (Gray, 1996). Acid mine drainage is principally associated with the mining of sulphide ores, especially sulphur, copper, zinc, silver, gold, lead and uranium. Elevated concentrations of iron and sulphate, a low pH and high concentrations of a wide variety of metals, depending on the host rock geology, characterise surface waters (Gray, 1998).

In acidified streams, invertebrate communities often have lower densities, fewer species, and a different taxonomic composition than sites with circumneutral water (Hall *et al.*, 1980; Townsend, Hildrew & Francis, 1983; Simpson, Bode & Colquhoun, 1985). A decline in the abundance of ephemeropteran taxa has been recorded frequently at pH 5.4 –5.7 (Feldman & Connor, 1992). Letterman & Mitsch (1978) found fewer invertebrate species in streams affected by coal mine drainage in western Pennsylvania with pH < 6 and Fe 3.6mgL^{-1} than in less acidic streams (pH > 6, Fe < 0.2 mg L^{-1}). Trichoptera, Ephemeroptera and Diptera dominated the control streams, whereas, Chironomidae (Diptera) were most tolerant of mine drainage.

In contrast, a small number of studies carried out on New Zealand West Coast streams have reported that a small diversity of aquatic invertebrates (including species of Plecoptera, Trichoptera, Ephemeroptera and Diptera) occur down to a pH of about 4 or even lower (Winterbourn & McDiffett, 1996; Winterbourn, 1998).

To explain changes in invertebrate assemblage composition, biologists have focussed on three hypotheses. Thus, an increase in hydrogen ion concentration may act directly by affecting the physiology of organisms, indirectly by increasing heavy metal concentrations which may be toxic to individuals, or indirectly by reducing primary production and/or bacterial decomposition and therefore the availability of food.

Low pH can affect different life cycle stages of organisms in different ways. For example, Burton *et al.* (1985) found that eggs of the snail *Physa heterotropha* were unable to develop at pH 4.0 whereas larval mayflies appear to be particularly sensitive to low pH

(Giberson & Mackay, 1991; Merrett et al., 1991; Feldman & Connor, 1992), especially during moulting (Tabak & Gibbs, 1991). Low pH may also limit the distribution of mayflies by inhibiting reproductive behaviour, and Sutcliffe and Carrick (1973) stated that adult female *Baetis* avoided laying eggs in acidic streams.

Low pH can also have direct toxic effects on aquatic invertebrates through the disruption of osmoregulation (Rankin & Davenport, 1981) and may result in severe losses of major ions (Na, Cl, Ca and K) in many animals (Rowe *et al.*, 1989). Hall et al. (1980) found that in a stream artificially acidified to pH 4 with sulphuric acid, invertebrates responded to low pH by drifting downstream before food supply could have been affected. This indicated that behavioural mechanisms can also be important in structuring invertebrate communities in culturally acidified streams (Hall *et al.*, 1980).

The epilithon represents an important food source for many acid stream invertebrates (Engleman & McDiffett, 1996) and therefore changes in its composition might be expected to influence this density and production. The rate of cellulose (plant fibre) decomposition in streams declines with pH below about 5.8 (Townsend *et al.*, 1983) and inhibition of organic layer formation can also occur. Sutcliffe & Carrick (1973) proposed that changes in food supply may be responsible for the impoverished invertebrate faunas found in streams of the English Lake District with pH < 5.7. Maurice et al. (1987) noted that the primary factor causing reduced rates of periphytic algal colonisation in acidified streams in the Upper Peninsula of Michigan was the elevated iron and aluminium concentrations and low nitrate and phosphorous concentrations. However, Hall et al. (1980) reported greater algal accumulation in artificially acidified streams due to reduced grazing pressure.

Streambeds covered by metal precipitates such as iron flocs tend to have impoverished and quite different communities than streams with elevated metal concentrations but no precipitate (McKnight & Feder, 1984). This may be partly due to the infilling of interstitial spaces, and partly due to the tendency of iron-rich sediments to consolidate. Iron deposits also reduce or eliminate the standing crop of periphyton, firstly by preventing its attachment to a stable substrate and secondly by inhibition of photosynthesis, thereby limiting growth (Penny, 1987).

In addition to surface waters, mine drainage can be expected to contaminate ground waters and the hyporheic zone where ground and surface waters mix (Fig 1.1). However, few ecologists (Nelson et al., 1993; Gray, 1996) have studied water quality within the hyporheic zones of acid mine drainage-affected streams, despite the "hyporheic zone" recently becoming a subject of general interest in furthering our understanding of lotic ecosystem structure (White, 1993).

By definition, the term "hyporheic zone" refers to the interstitial habitat within a streambed, bounded by the surface water of the stream above, and by true groundwater below (Fraser *et al.*, 1996).

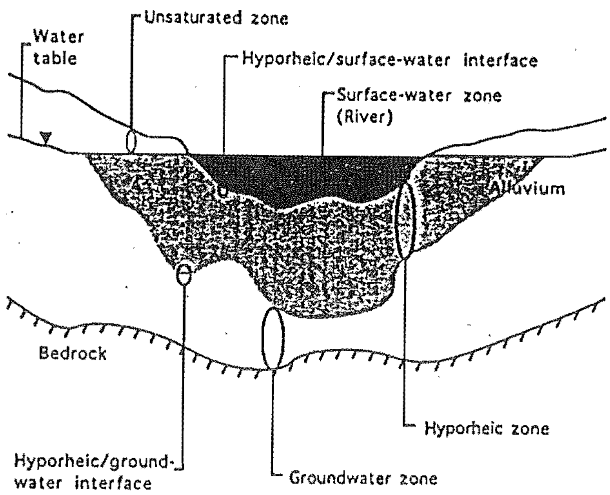


Fig 1.1 Conceptual model of a river valley showing the spatial relationships among the groundwater, hyporheic, and surface-water zones. (Source: Fraser *et al.*, 1996).

Typically, it is limited to an area no more than a few meters below the river bed and beyond the river margins (Williams & Hynes, 1974), but its extent varies from stream to stream in response to a suite of environmental factors (Triska *et al.*, 1989). These include, grain size, porosity of the bed sediments, physical extent of alluvial sediment and physico-chemical conditions (Triska *et al.*, 1989; Ward, 1992; Scarsbrook, 1995; Scarsbrook & Halliday, 1996). Streams flowing over bedrock or unstable sandy beds often lack a well developed hyporheic community (or zone), due to the absence of interstitial spaces (Gray & Fisher, 1981; Boulton & Suter, 1986).

Behavioural responses are important in determining the survival of animals exposed to deleterious water quality (Brakke *et al.*, 1994). For example, several studies have shown that fish seek refugia based on better water quality, often in spite of large population densities or shortages of food at these sites (Rosseland & Staurnes, 1994). The existence of refugia might be crucial for survival, under conditions of fluctuating acidity (Brakke *et al.*, 1994) and interstitial and hyporheic environments of streams may be places that provide refugia for invertebrates in acid mine-drainage affected streams. Gray (1996) found that iron and conductivity values were slightly lower and pH higher in the hyporheic zone of streams affected by the Avoca mines in County Wicklow, Ireland. However, Nelson *et al.* (1993) reported that relative to surface water, hyporheic waters in the upper Arkansas River in central Colorado, had higher concentrations of certain metals and that both surface and hyporheic waters had similar invertebrate communities. Thus, surface and hyporheic sediments of acid mine drainage-affected streams were dominated by Diptera, including Chironomidae, and had similar levels of taxonomic richness.

The objective of my study was to investigate the effects of acid mine drainage on the physico-chemical conditions of surface and hyporheic receiving waters and to examine the relationship between these conditions and invertebrate community structure, and epilithic algal production. My research focussed on the following questions:

1) Do the taxonomic composition and abundance of benthic and hyporheic invertebrate assemblages reflect differences in the physico-chemical environments of the streams ?
2) Do streams affected by acid mine drainage have distinct hyporheic and surface substrate invertebrate communities ?
3) Do algal production and epilithic community respiration differ between streams acidified by mine-drainage to varying degrees ?

In Chapter 2 a brief history of mining in Reefton and the environment of the study area is described and details are given of the ten study sites. Chapter 3 presents results of physico-chemical conditions of the streams. Seasonal changes and affects of varying degrees of acidity on epilithon are also described here. Chapter 4 describes the distribution, abundance and diversity of stream invertebrates both among surface substrates and in the hyporheic zone in relation to physico-chemical conditions. The tolerance levels of three New Zealand stream invertebrates, *Deleatidium* sp., *Zelandobius confusus* and *Pycnocentrella eruensis*, to low pH and elevated iron concentrations, as indicated by 96 hour toxicity tests, are also considered in this chapter. Finally, in Chapter 5, my findings are summarised and considered in relation to those obtained in other New Zealand and overseas stream studies of acid mine drainage-affected streams.

Chapter Two

STUDY AREA

BACKGROUND

Ten study sites were chosen for the sampling programme. The sites were associated with the Murray Creek Goldfield (Fig 2.1) and Alborns Coal Mine (Fig 2.2) in the Reefton area, North Westland. They were chosen to reflect a range of pH values. The sites are described in detail below, along with general descriptions of the geology, climate, history, and vegetation of the study area.

Geology

Reefton (approximately 42°7' S, 171°52' E, N.Z.M.S. Sheet S38, Reefton) lies in Victoria Forest Park, just at the point where the Inangahua River emerges from its gorge (Peat, 1989).

The geological history of the Reefton area is long and complicated and was first detailed by Henderson (1917) and later by Suggate (1957). The Reefton Goldfield, or the Reefton Mineral Belt, an area 30 – 40 km long by 15 km wide, is dominated by basement rocks of the Greenland Group, predominantly argillites and greywackes (Weber 1993). The Greenland Group is regarded as the basement lithology within the Reefton area and is overlain by lower Devonian fossiliferous mudstones, limestones and quartzite (Reefton Group).

During the late Devonian period, bodies of molten magma called "plutons" intruded upon the strata of the Greenland Group. Early workers (e.g., the Murray Brothers) advocated that these plutons provided the source of the mineralising fluids for the Reefton Goldfield.

Within the Murray Creek Goldfield three distinct geological formations occur: the hard greenish-grey sandstones and mudstones of the Greenland Group surround the gold-bearing quartz veins, the fossil-rich Reefton Group rocks, and the Quartzose coal

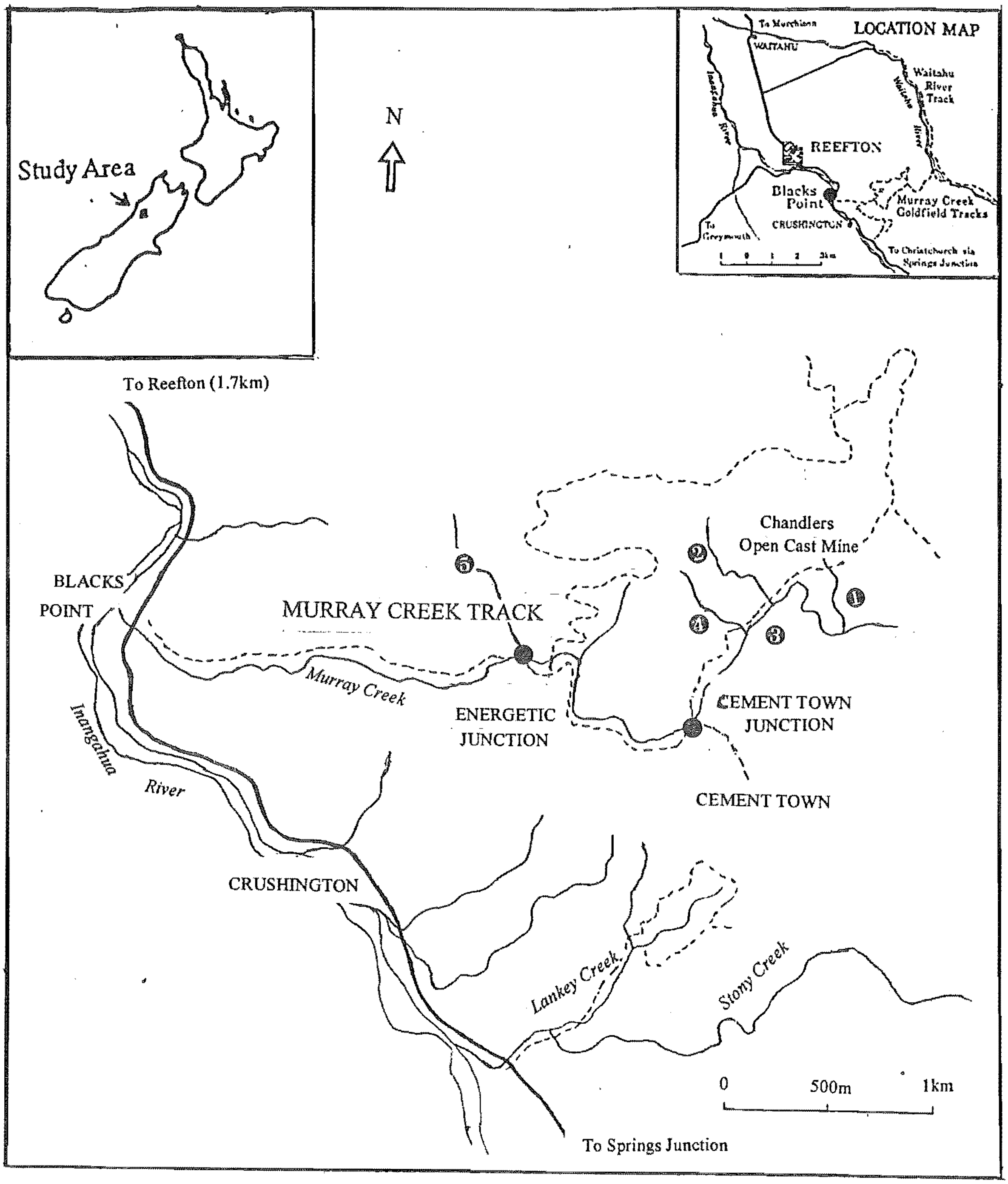


Fig 2.1 Location of study sites associated with the Murray Creek Goldfield (represented by the numbers 1 to 5) (source: Department of Conservation, 1990).

B

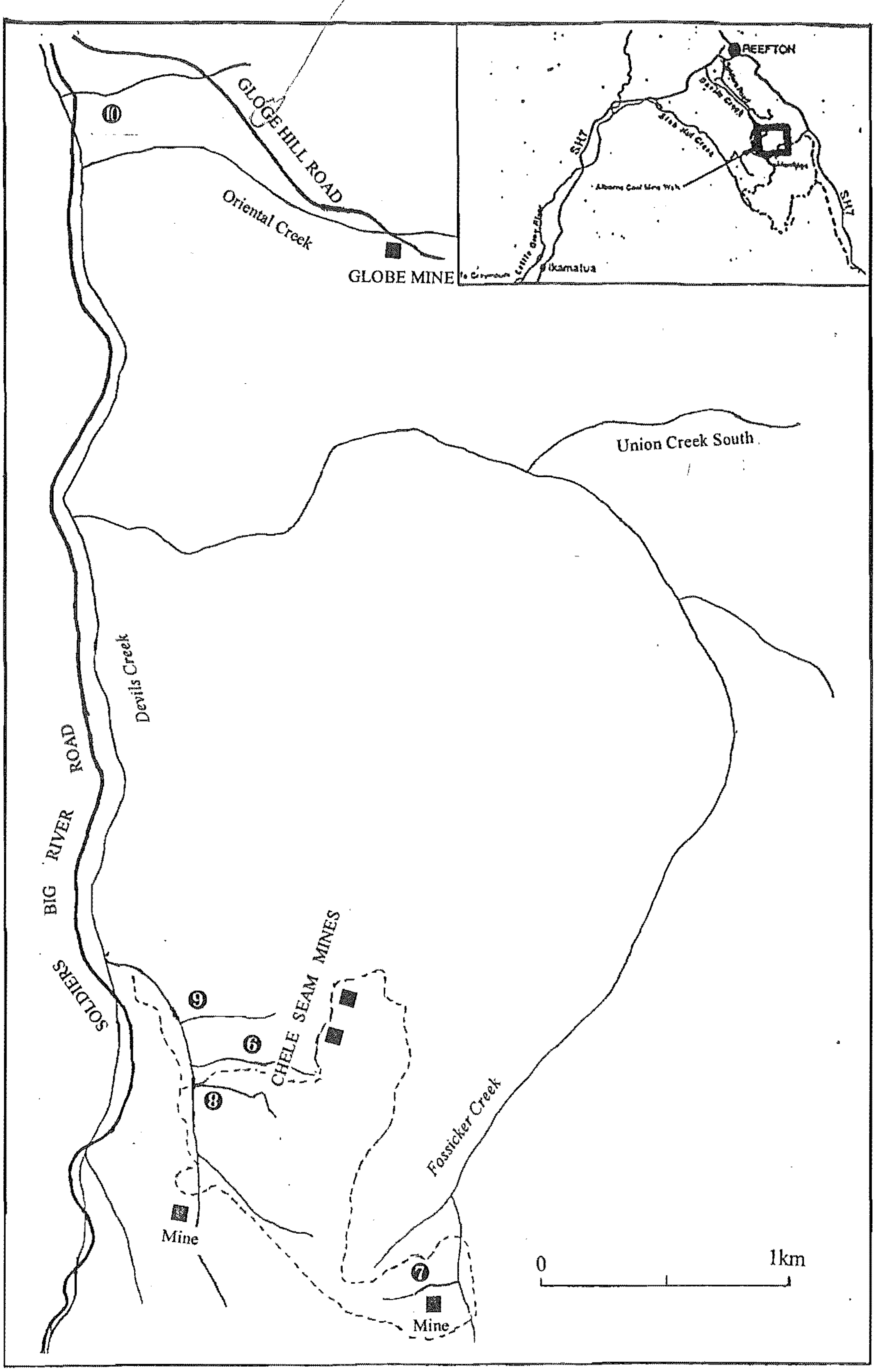


Fig 2.2 Location of study sites associated with Alborns Coal Mine (represented by the numbers 6 to 10) (source: Department of Conservation, 1987).

measures (Department of Conservation, 1990). Reefton group rocks lie along a half kilometre wide band running south from near Chandlers opencast mine (Fig 2.1.) to the Inangahua River. The most recent formations in this area are a sequence of tertiary sandstone beds and coal measures dating back to the Eocene period (about 38 - 45 million years ago) (Weber, 1993).

Alborns Coal Mine lies within the southern section of the Devils Creek catchment, and is part of the Reefton Coalfield. Here, Greenland Group rocks are overlain by tertiary coal measures, typically referred to as the Brunner Coal Measures (Suggate, 1957).

History

There was phenomenal interest in Reefton last century on account of its proximity to rich deposits of gold in a quartz reef (hence "Reef Town") (Peat, 1989). The first gold was discovered near the Buller River in 1859 (Henderson, 1917), while the first alluvial gold was sluiced from the Victoria Range foothills in 1866 by the Murray Brothers. However, the initial wave of real gold fever occurred in Reefton in late 1870 when rich, goldbearing quartz reefs were discovered in Murray Creek by a prospector named Patrick Kelly (Peat, 1989). In an area roughly six kilometres square, more than two hundred claims were pegged out by various companies before reserves of gold were exhausted in the 1930s (Department of Conservation, 1990). After more than 80 years, the goldmining era came to a close in 1951 (Wright, 1992).

Two other industries boomed with the onset of goldmining in the Reefton area: timber milling and coal mining (Cowie, 1980). The resources these industries produced, along with a plentiful water supply, were largely responsible for the success of gold mining. Trees were felled to provide sleepers for tramways and fluming for water races (Wright, 1992) and nearby coal seams were mined to provide fuel for the gold mining operations. Good examples of such coal mines are Cement Town, Chandlers opencast mine (Fig 2.1) and Alborns underground coalmine (Fig 2.2). Chandlers was opened in 1888 and supplied the Reefton domestic market while also fuelling the Murray Creek Goldfield (Fig 2.1) until the early 1960s (Department of Conservation, 1990). Coal from Alborns Coal Mine initially supplied goldmining ventures in Devils Creek and on Globe Hill (Fig 2.2) (Department of Conservation, 1987).

Climate

Weather patterns in the Reefton area are strongly influenced by the Paparoa Range which shelters Reefton from the full impact of the West (or "Wet") Coast weather (Wright, 1992). The prevailing westerlies are associated with considerable rain, while warm wet nor'westerlies bring the heaviest rain especially in spring and early summer (Wright, 1992).

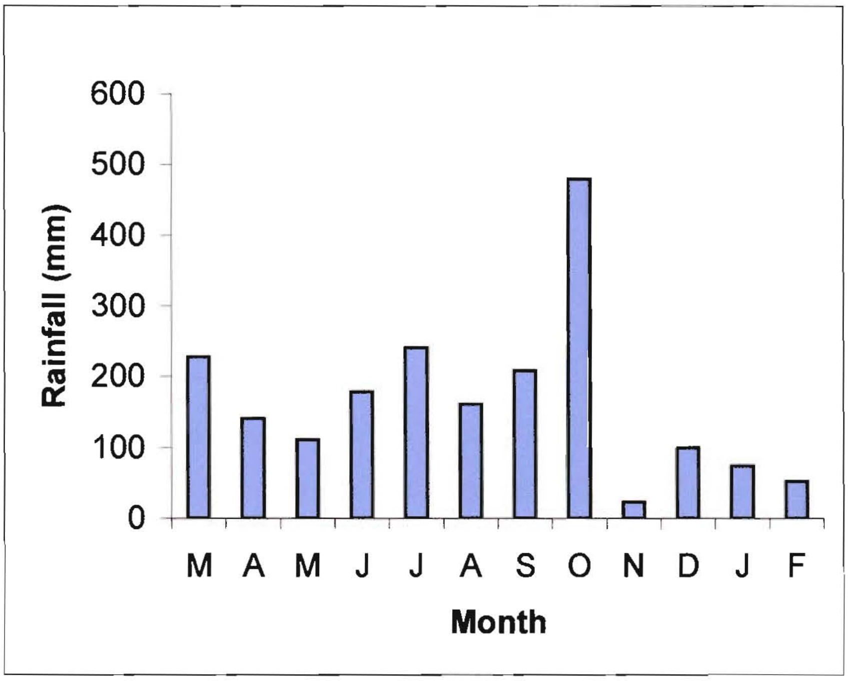
During the 12 months of my study, precipitation (rain and snow) recorded at the Reefton Mine Weather Station (lat 42°117'S, 171°86'E) was 1962 mm, slightly above the average rainfall for the last ten years of 1885 mm. The highest monthly rainfall recorded during the observation period (March 1998 – February 1999) was 479 mm in October 1998 (Fig 2.3a). This was the highest monthly rainfall recorded at the Reefton Mine Weather Station for the past 10 years. The fact that the lowest rainfall recorded during the study period (about 22.8 mm), occurred the following month (November 1998) reflects the variability that is a feature of the climate of the West Coast (NIWA climate records, pers. comm.).

During the study period, the maximum temperature recorded at the Reefton Mine Weather Station was 26.6°C, in February 1999, and the minimum temperature was 1.4°C in August 1998 (Fig 2.3b) (NIWA climate records, pers. comm.).

Vegetation

As a result of the diverse geology and soil types of the region, vegetation varies greatly. Beech trees (*Nothofagus* sp.) and mixed podocarps comprise the forest canopy in most areas (Wright, 1992). Much of the forest that was cleared for early mining operations is regenerating in red (*Nothofagus fusca*), silver (*Nothofagus menziesii*) and mountain beech (*Nothofagus solandri var. cliffortioides*), although kaikawaka (*Libocedrus bidwillii*), rimu (*Dacrydium cupressinum*), mountain toatoa (*Phyllocladus alpinus*), rata (*Metrosideros robusta*) and other hardy species are also present (Department of Conservation, 1987). Some extremely infertile or poorly drained sites have reverted to pakihi (stunted scrub such as manuka (*Leptospermum scoparium*), bracken (*Pteridium aquilinum* var. *esculentum*) and gorse (*Ulex europaeus*)) (Wright, 1992). The relatively high annual rainfall also ensures a generous covering of mosses and lichens.

a)



b)

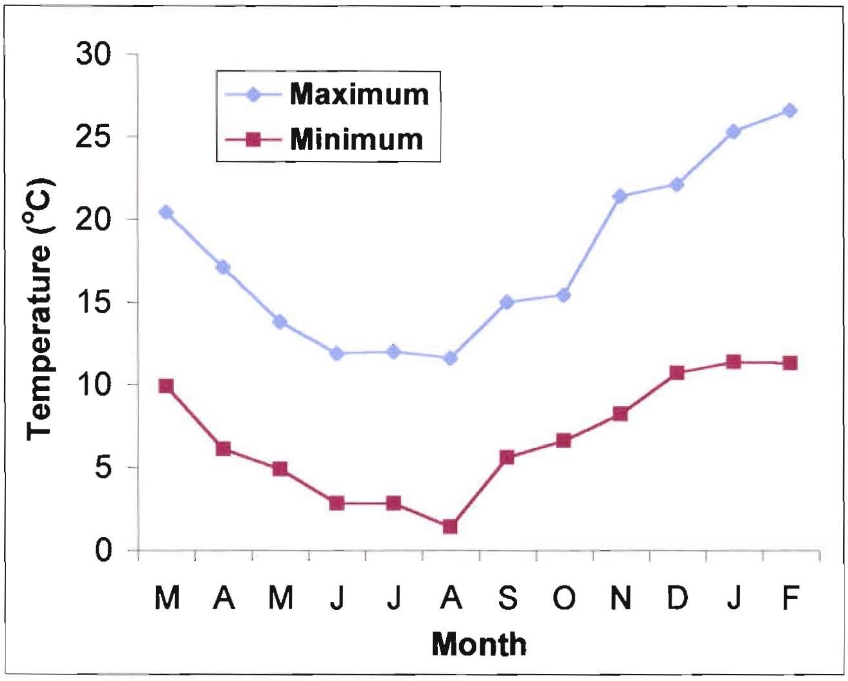


Fig 2.3 a) Rainfall recorded at the Reefton Mine Weather Station monthly from 1 March 1998 to 28 February 1999.

b) Maximum-minimum air temperatures recorded at the Reefton Mine Weather Station from 1 March to 28 February 1999.

STUDY SITES

Study sites were selected to reflect a range of physico-chemical conditions (acidic – neutral). Streams had similar canopy (i.e., light) conditions, mostly shaded but all sites received partial sunlight, altitude, geology and were of a comparable size (width and depth) (Table 3.1). Sites were mainly shallow (> 30 cm) with riffles predominating each 50 m stream reach.

Site 1. (Fig 2.1) (Plate 2.1a & b)

Site 1 drained a tailings pond at the former site of Chandlers opencast mine (pH < 4). The vegetation adjacent to this site consisted mainly of manuka and gorse with a few scattered mountain beech trees. Stream slope was gentle (1 - 2°) and the substrate was dominated by small and medium sized cobbles (64 – 256 mm) (Wentworth classification, Cummins, 1964), with scattered coal deposits also apparent. Stream channel width averaged 0.5 – 1.5 m but in early January 1999, the stream dried up completely.

Site 2. (Fig 2.1) (Plate 2.2a)

Site 2 was a tributary of Murray Creek running alongside Chandlers opencast mine (pH > 5.0). The slope of the stream was gentle and stream channel width was about 1 m. Bed materials were predominantly moderate to large pebbles (4 – 64 mm) and cobbles interspersed with occasional boulders (> 256 mm diameter), and appeared to be very stable. Retention of leaf debris from the surrounding beech forest was relatively high. Small amounts of algae were observed on stone surfaces throughout the duration of the study, and a seasonal bloom occurred when low flow conditions occurred in summer (Dec 1998 – Feb 1999) (Fig 2.3a). Adjacent stream vegetation consisted of beech forest, manuka and gorse. Coal deposits were observed in the stream and on stream banks surrounding Sites 1 and 2.

Site 3. (Fig 2.1) (Plate 2.2b)

Site 3 was located on Murray Creek, a tributary of the Inangahua River. Stream width was about 2 m with several deep pools included within the sampling reach. Streambed materials were extremely heterogeneous, and consisted of unsorted boulders and cobbles of varying sizes. Fine gravel and sand (< 2mm) often accumulated in pools

a)

b)

Plate 2.1

a) Tailings pond at former site of Chandlers opencast mine.

b) Site 1.

a)

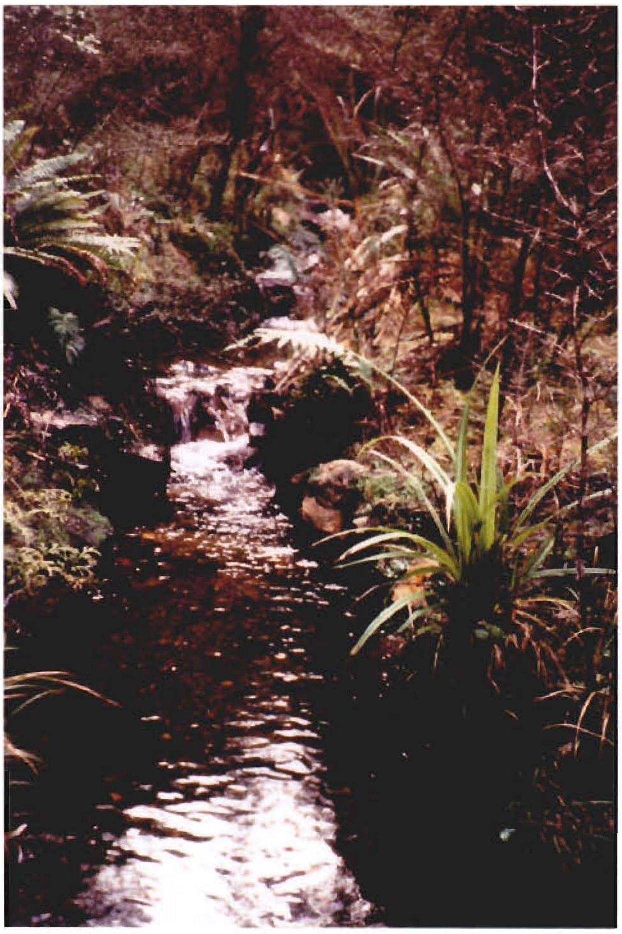


Plate 2.2

a) Site 2.

b) Site 3 with orange iron flocs covering bed substrates.

b)

behind small debris jams but were scoured from the system during flood events, especially in July and October 1998 (Fig 2.3a). Throughout the duration of the study orange iron flocs covered bed substrates. This covering increased during periods of low flow.

Site 4. (Fig 2.1) (Plate 2.3a)

Site 4 was about 1 m wide and was a tributary of Murray Creek (pH > 5.0). Stream slope was gentle and became steeper further upstream from the sampling point. The site was surrounded by beech forest and like Site 3, leaf litter retention appeared to be quite high. The substrate consisted of sandy deposits and evenly sorted pebbles and cobbles, with a few larger boulders present.

Site 5. (Fig 2.1) (Plate 2.3b)

Site 5 was a tributary of Murray Creek with circumneutral water and was used as a control site. Vegetation on the stream bank was mainly beech forest. The slope of the stream was relatively steep (> 5^{o}) and unstable. Stream channel width averaged 0.5 – 1 m, increasing to 2 m during periods of high flow. During the study period, the course of the stream channel within the bed changed several times. The substrate consisted of sandy deposits and well-sorted stones, varying in size from small pebbles to large boulders.

Site 6. (Fig 2.2) (Plate 2.4a)

Site 6 was a small forest stream flowing into Progress Creek, a major tributary of Devils Creek. The dominant canopy species was silver beech, which sheltered a ground cover of *Sphagnum* moss, bracken and other ferns. Stream slope was gentle (1 – 2^{o}) and small to medium sized pebbles dominated the bed. Streambed width was 0.5 – 1 m. Several times during the 12 months of the study the streambed was overlain with a heavy deposit of silt and mud. This may have originated from a precipitate forming from the mine drainage waters.

Site 7. (Fig 2.2) (Plate 2.4b)

Site 7 was a tributary of Fossickers Creek, a first order forest stream. Slightly

a)

Plate 2.3

a) Site 4.

b) Site 5 with tiles in place for epilithic colonisation experiment.

b)

a)

Plate 2.4

a) Site 6.

b) Site 7 with tiles in place for epilithic colonisation experiment.

b)

stunted silver beech trees along with some kamahi (*Weinmannia racemosa*) dominated the adjacent vegetation. Stream slope was gentle (1 – 2°), and pebbles and cobbles dominated the substrate. Streambed width was about 0.5 – 1 m. As at Site 6, the streambed of Site 7 was overlain with a heavy deposit of silt and mud.

Site 8. (Fig 2.2) (Plate 2.5a)

Site 8, a brown water stream, was also a tributary of Progress Creek. The site was surrounded by manuka and tussock with a scattering of silver beech trees. Stream slope was gentle (1 – 2°) and the stony streambed was covered in fine silt throughout the study. Stream channel width was about 0.5 m. The brown waters within Progress Creek are presumably related to drainage from pakahi in their catchments.

Site 9. (Fig 2.2) (Plate 2.5b)

Site 9 was yet another tributary of Progress Creek. Scrubby manuka, grasses and some small silver beech trees dominated the surrounding vegetation. Stream substrate was predominately moderate to large pebbles with some cobbles interspersed and stream width was 0.5 – 2 m. A deep pool (> 1 m) was included in the sampling reach.

Sites 6 to 9 received acid mine drainage and all had pH < 4.

Site 10. (Fig 2.2) (Plate 2.5c)

Site 10 was a tributary of Devils Creek and was considered a control site as it had circumneutral water. Streambed materials were unsorted boulders and cobbles of varying sizes. In places, wood debris had accumulated in the channel, giving the stream a staircase-like appearance. Stream width was 0.5 – 1.5 m. The stream channel changed course during large floods, which occurred in July and October 1998 (Fig 2.3a). Algal mats covered stones in some low velocity areas and pools within this stream.

b)

Plate 2.5

a) Site 8.

b) Site 9.

c) Site 10.

Chapter Three

PHYSICO-CHEMICAL CONDITIONS and EPILITHON

INTRODUCTION

Natural streams exhibit wide variations in water chemistry as a result of differences in the geology, soils, vegetation and run-off characteristics of their catchments (Stewart 1993). Such factors as season, time of day, locality and depth may also vary the chemical composition of river water. Studies into the effects of acidification on freshwater ecosystems in the Northern Hemisphere by acid rain, have highlighted the deleterious effects of low pH and associated chemical changes on aquatic life (Haines, 1981).

Although acid precipitation is largely absent from New Zealand, the West Coast of the South Island has many acid streams, both natural brown waters and others affected by acid mine drainage (Winterbourn & McDiffett, 1996). Acid mine drainage can cause significant environmental degradation by impacting on the chemistry and biology of waters. Several studies have found strong relationships between the chemical nature of stream water and distributions of benthic invertebrates in streams affected by acid mine drainage, both in New Zealand (e.g., Winterbourn & McDiffett, 1996; Winterbourn, 1998), and overseas (e.g., Hargreaves, Lloyd & Whitton, 1975; Scullion & Edwards, 1980).

Where acid mine drainage is persistent and extreme, the pH of affected streams may be pH < 3 and the water may have very high conductivity and low alkalinity. Low pH is often coupled with high concentrations of certain metal ions, for example, iron and aluminum, as well as sulphate, phosphate and nitrate (Scullion & Edwards, 1980; Engleman & McDiffett, 1996; Winterbourn & McDiffett, 1996).

In addition to surface waters, mine drainage can be expected to contaminate ground waters and the hyporheic zone where ground and surface waters mix. However, to date few ecologists appear to have studied water quality within the hyporheic zone of acid mine drainage-affected streams, in spite of the reported importance of this zone to in-stream processes and invertebrate ecology (Triska *et al.*, 1989). Nelson *et al.* (1993) observed that relative to surface water, hyporheic waters in the upper Arkansas River in central Colorado contained higher concentrations of certain metals, suggesting that mine drainage could have serious effects in the hyporheos.

Other important stream biota that could be adversely affected by acid mine drainage are algae, fungi and bacteria, which along with the slimes they produce and fine particulate material form the stone surface layers (epilithon) in streams (Lock, 1981). The epilithon represents an important food source for many stream invertebrates (Rounick & Winterbourn, 1983; Winterbourn *et al.*, 1985; Engleman & McDiffett, 1996) and therefore changes in its composition might be expected to influence density and production of benthic invertebrates.

Most studies into the effects of acidification on epilithon in the Northern Hemisphere have demonstrated an increase in algal biomass with decreasing pH (Hall *et al.*, 1980; Müller, 1980; Mulholland *et al.*, 1986). However, Maurice et al. (1987) recorded reduced periphytic biomass in experimentally acidified Michigan streams, apparently as a result of elevated metal (aluminum and/or iron) concentrations and lower nutrient availability.

The aim of the work reported in this chapter was to investigate the physico-chemical composition of surface and hyporheic waters, and epilithon. The research focused on the following questions:

1) How do mine-affected and control sites differ with respect to physico-chemical conditions ?
2) Is surface water chemically similar to that in the hyporheic zone ?
3) Does water chemistry change with respect to seasonal and/or other conditions (e.g., stream flow) ?
4) Does epilithon differ between streams acidified by mine drainage to varying degrees ?

METHODS

This part of the field programme involved the sampling of selected physico-chemical parameters, algal production and epilithic community respiration at regular intervals between March 1998 and February 1999.

Physical measurements

Stream channel stability was assessed for the sampled reach of each stream using the method of Pfankuch (1975), which has been used in a number of New Zealand studies (e.g., Collier & Winterbourn, 1987; Winterbourn & Collier, 1987; Graesser, 1988; Chadderton, 1990; Brown, 1998).

The method involves the evaluation of 15 features of the upper banks, lower banks and stream bottom in accordance with stated criteria (Appendix I). Selected scores for all criteria were summed to give a channel stability index, which is interpreted as follows: < 38 = excellent (very stable); 39 – 76 = good; 77 – 114 = fair; > 114 = poor (highly unstable). The rating indicates the capacity of a stream reach to resist bed and bank material detachment and to recover from potential hydrological changes (Pfankuch, 1975). Scores for the bottom component of the index were also considered separately since they provide a direct measure of the stability of benthic invertebrate habitat that correlates well with several hydrological measures of stream stability (Death & Winterbourn, 1994).

Stream water temperature was recorded on 8 occasions with a calibrated maximum-minimum thermometer, held about 5 cm from the stream bottom.

Stream channel width and depth were measured at 5 points along each reach using a metre rule. Discharge was determined by measuring current velocity (the time taken for a cork to travel a fixed distance of 1 m) multiplied by mean cross-sectional area at five points.

Chemical measurements

Stream water pH, alkalinity and conductivity were measured twice each season (total 8 measurements), whereas all other chemical variables were measured once per season. Water samples were collected from each stream and stored in acid-washed polyethylene bottles for measurement of pH and conductivity (within 12 hours), alkalinity, nitrate-nitrogen, reactive phosphate, total dissolved aluminium, and total iron within three days. Water samples were kept dark and cool prior to analysis.

Water samples were also collected seasonally, from the hyporheic zone at each site. Up to 5 litres of water were pumped from stainless-steel wells (diameter 16 mm) hammered (to a depth of 30 cm) into the stream bed (see Boulton et al., 1992). Water samples were stored as described above and the same analyses were made on them as for surface water samples.

pH was measured using a Metrohm Herisau E488 meter. Conductivity (at 25°C) was measured in $\mu S\ cm^{-1}$ with a Hanna H1 8333 conductivity meter. Alkalinity was determined by titration with 0.01N HCl (Mackereth, 1963). Concentrations of nitrate-nitrogen, reactive phosphate, total dissolved aluminium and total iron were determined on filtered (0.2 μm pore size) subsamples using Hach DR/2000 spectrophotometric procedures (Hach, 1992).

Nitrate-nitrogen and reactive phosphate were measured since their availability can limit the production of epilithic algal biomass including that in acid streams (Maurice et al., 1987). Aluminium was measured because it is known to be toxic to many insects at elevated concentrations which typically occur in acid waters, (Sutcliffe & Hildrew, 1989; Collier & Winterbourn, 1987; Winterbourn, 1998), while various concentrations of dissolved iron have also been found to be toxic to invertebrates and are also elevated in some mine drainages in New Zealand (Winterbourn, 1998).

Epilithon

Biomass, primary production and community respiration of stone surface biofilms (epilithon) were examined using artificial substrates incubated in six streams between March 1998 and February 1999.

The substrates used were red brick tiles (20 x 10 x 1.5 cm) pegged into the stream channel. Six sites (Sites 1, 2, 5, 6, 7 and 10) (Figs 2.1 and 2.2) with similar canopy (i.e., light) conditions (essentially closed) were selected for this part of the study. All tiles were placed in relatively shallow (< 10 cm depth) riffles at each site. Five tiles were incubated in each of the six streams and were removed after approximately two months. Colonisation experiments were repeated seasonally.

Following collection, tiles were used either to measure primary production and community respiration (i.e., respiration of total epilithon i.e. algae, bacteria, fungi) (2 tiles) or assayed for chlorophyll a (3 tiles) as described below. Primary production of epilithic algae was measured by incubating tiles in sealed 500 ml plastic containers (with transparent lids), of water from the individual stream in a temperature controlled room at 5 .°C, which approximated the temperature of ambient stream water (± 2 °C). The temperature control room was on a 12 hour light:12 hour dark cycle (fluorescent lighting). Two control jars were run concurrently with the experimental containers and contained stream water with a clean (i.e. uncolonized) tile. After both the light and dark incubation periods, oxygen concentrations were measured with a YSI model 54 oxygen meter and electrode. Respiration rates were calculated as mg O_2 used or produced relative to controls per cm^2 of tile surface per hour.

To obtain an estimate of epilithic algal biomass, the remaining 3 tiles were used to measure chlorophyll a (chl a). In the laboratory, tiles were placed individually in 500 ml plastic containers and covered with known volumes of 95 % ethanol to extract chl a from attached algae. Lids were placed on the containers, which were kept in the dark at 7 °C overnight. Absorbancies (665 and 750 nm) of extracts were measured on a Contron-Uvicon Spectrophotometer. Concentrations of chl a were calculated with the formula below and expressed per unit area of tile surface.

$$\text{Chlorophyll a } (\mu g\ cm^{-2}) = 12 * \frac{\text{Abs } (665 - 750) * \text{ml ethanol}}{\text{area } (cm^2)}$$

reference ?

Statistical Analysis

Physical and chemical data were analysed using STATISTIX. Kruskal-Wallis one-way nonparametric ANOVAs were performed to determine whether differences in chemical variables occurred among streams. Tukey multiple comparison *a posteriori* tests were carried out following a significant ANOVA to determine where significant differences lay. Spearmans rank correlations were also performed on physico-chemical data to determine whether variables were intercorrelated. Log transformed epilithon data (biomass and community respiration) were analysed using a two-way ANOVA to see if there were significant differences between site and seasons.

RESULTS

Physical conditions

Physical features of the 10 study sites measured between March 1998 and February 1999 are summarized in Table 3.1.

significant but not strong.

Surface water temperatures ranged from 6 to 16 °C and were highest in summer when temperatures ranged from 9 °C (Site 9) to 14 °C (Site 8). During periods of high flow, water temperatures decreased and the range of temperatures between sites was also reduced. A linear regression showed that temperature was significantly and negatively correlated with stream discharge ($r^2 = 0.149$, $P < 0.05$). Mine affected sites had slightly higher temperatures than control sites, the exception being Site 3, which had the lowest mean temperature of 7.1 °C. This may have reflected the larger size of this stream. Site means for width ranged from 41 cm to 2.12 m, while depth means ranged from 10 cm to 53 cm (Table 3.1). Stream discharge varied considerably among sites. Site 3 had the highest mean discharge ($0.7\ m^3\ s^{-1}$), while the lowest discharge ($0.009\ m^3\ s^{-1}$) was recorded at Site 8. Discharge was greatest during periods of high rainfall (Fig 2.3a). The distance of each site from a mine is also shown

is this surprising ?

Table 3.1 Physical characteristics of the ten study sites (mean values with ranges in parentheses).

Site	Width (m)	Depth (m)	Discharge (m^3s^{-1})	Temperature (°C)	Total Stability	Bottom Stability	Distance from mine (m)
Site 1	0.41 (0.25 - 1.01)	0.16 (0.05 - 0.41)	0.02 (0.00012 - 0.04)	8.2 (6 - 13)	76 (good)	32	50
Site 2	0.88 (0.42 - 1.49)	0.16 (0.03 - 0.39)	0.04 (0.01 - 0.08)	7.8 (6.5 - 11)	76 (good)	36	200
Site 3	2.12 (1.20 - 2.90)	0.53 (0.20 - 1.00)	0.7 (0.13 - 1.45)	7.1 (6.5 - 9)	99 (fair)	41	700
Site 4	1 (0.88 - 1.20)	0.13 (0.02 - 0.33)	0.04 (0.01 - 0.08)	8.2 (6.5 - 13)	79 (fair)	29	750
Site 5	0.68 (0.30 - 0.83)	0.1 (0.06 -0. 20)	0.04 (0.013 - 0.06)	7.2 (6.8 - 10)	107 (fair)	42	2500
Site 6	0.67 (0.41 - 1.20)	0.14 (0.03 - 0.31)	0.14 (0.009 - 0.03)	8.2 (7 - 11)	73 (good)	24	10
Site 7	0.5 (0.34 - 0.71)	0.11 (0.04 - 0.29)	0.01 (0.003 - 0.014)	8.5 (7 - 10)	80 (fair)	29	50
Site 8	0.43 (0.22 - 0.98)	0.15 (0.02 - 0.53)	0.009 (0.003 - 0.02)	10.6 (7.3 - 16)	72 (fair)	29	400
Site 9	0.65 (0.25 - 0.96)	0.38 (0.15 - 0.63)	0.05 (0.014 - 0.09)	8.1 (6 - 11)	81 (fair)	34	200
Site 10	1.2 (0.68 - 1.50)	0.23 (0.10 - 0.41)	0.16 (0.13 - 0.2)	7.7 (6.4 - 10)	84 (fair)	34	2000

in Table 3.1 and ranged from 10 m to 2.5 km. As one would expect, the distance of mine affected sites to the closest mine was a lot shorter than the distance from the control sites to a mine.

Pfankuch channel stability scores ranged from 72 to 107 (Table 3.1) at the 10 sites, and were indicative of good to fair stability (Pfankuch, 1975, Appendix I). Site 8 had the highest channel stability (score = 72), whereas Site 5 had the most unstable score. This was probably associated with the fact that the stream at Site 5 was slightly steeper than the other streams (see Chapter 2). Site 6 had the most stable stream bed as indicated by the Pfankuch bottom score (24). Scores of the other sites ranged from 29 - 42 (Table 3.1). In general, bottom scores corresponded with overall stability scores ($r_s = 0.73$, $P < 0.05$). The control sites (Sites 5 and 10) were two of the least stable, reflecting the fact that both streams changed course several times during the study period.

Chemical conditions

Surface stream waters

Surface water chemical conditions at the 10 study sites measured between March 1998 and February 1999 are summarised in Table 3.2 and in Figs 3.1 to 3.5.

Mean pH of surface water ranged from 3.2 at Site 6 to 6.9 at Site 10 (control site). However, during the study period the pH at all sites fluctuated over a range of 0.4 to 1.0 unit (Table 3.2). Differences recorded in pH between acidic and control sites were highly significant ($P < 0.001$). pH did not appear to display any seasonal patterns, although lowest readings for most streams were recorded during spring (September to November) (Fig 3.1a & b). A linear regression showed that pH was significantly and negatively related with discharge ($r^2 = 0.224$, $P < 0.05$). As discharge increased, stream water pH decreased (i.e., became more acidic).

Conductivity of the 10 streams ranged from 29 $\mu S\ cm^{-1}$ to 747 $\mu S\ cm^{-1}$ and was greatest at the most acidic site (Site 6) (Table 3.2). It did not appear to display any seasonal trend although lowest readings were recorded in November at all but two sites (Sites 7 and 9), and highest values occurred in January (Fig 3.2a & b). A linear regression showed that stream conductivity was significantly and negatively associated with discharge ($r^2 = 0.314$, $P < 0.001$). Strongly significant differences ($P < 0.001$) were observed in conductivity values

Table 3.2 Stream water chemistry at the ten study sites (mean values with ranges in parentheses).

Site	pH	Conductivity ($\mu S_{25}\ cm^{-1}$)	Alkalinity ($mg\ L^{-1}$)	Nitrate - Nitrogen ($mg\ L^{-1}$)	Reactive Phosphate ($mg\ L^{-1}$)	Total Iron ($mg\ L^{-1}$)	Total dissolved Aluminium ($mg\ L^{-1}$)
Site 1	3.3 (3.0 - 3.6)	266 (220 - 303)	0.16 (0 - 0.5)	0.08 (0.04 - 0.11)	0.05 (0.02 - 0.14)	1.16 (1.08 - 1.33)	1.29 (0.57 - 1.9)
Site 2	5.5 (5.1 - 5.9)	56 (41 - 73)	19 (15 - 23)	0.07 (0.03 - 0.1)	0.04 (0 - 0.08)	0.23 (0.1 - 0.52)	0.02 (0 - 0.05)
Site 3	4.9 (4.5 - 5.3)	70 (59 - 78)	8 (5 - 16)	0.02 (0 - 0.04)	0.03 (0.01 - 0.07)	1 (0.13 - 2.81)	0.05 (0.04 - 0.06)
Site 4	5.7 (5.5 - 5.9)	48 (29 - 63)	19 (13 -24)	0.05 (0.02 - 0.08)	0.05 (0.01 - 0.07)	0.2 (0 - 0.58)	0.028 (0.02 - 0.03)
Site 5	6.8 (6.5 - 7.1)	112 (96 - 146)	46 (35 - 62)	0.06 (0 - 0.1)	0.53 (0.04 - 0.7)	0.02 (0 - 0.08)	0.005 (0 - 0.02)
Site 6	3.2 (2.9 - 3.4)	565 (398 - 747)	0.2 (0 - 0.5)	0.06 (0.02 - 0.08)	0.05 (0.01 - 0.1)	2.35 (1.8 - 3.5)	5.83 (5.1 - 6.9)
Site 7	3.4 (3.1 - 3.9)	465 (302 - 545)	0.94 (0 - 3)	0.05 (0.03 - 0.07)	0.1 (0 - 0.2)	0.79 (0.17 - 1.61)	1.43 (1.2 - 1.8)
Site 8	3.3 (3.0 - 3.5)	112 (62 - 159)	0.83 (0 - 2.5)	0.003 (0 - 0.01)	0.12 (0.02 - 0.23)	1.8 (0.69 - 3.22)	0.24 (0.17 - 0.35)
Site 9	3.7 (3.1 - 4.1)	162 (73 - 248)	5 (1 - 12)	0.02 (0 - 0.07)	0.04 (0 - 0.07)	0.93 (0.68 - 1.27)	0.65 (0.3 - 1.05)
Site 10	6.9 (6.8 - 7.1)	71 (61 - 79)	30 (23 - 45)	0.06 (0.02 - 0.1)	0.15 (0.04 - 0.23)	0.04 (0.03 - 0.04)	0.003 (0 - 0.01)

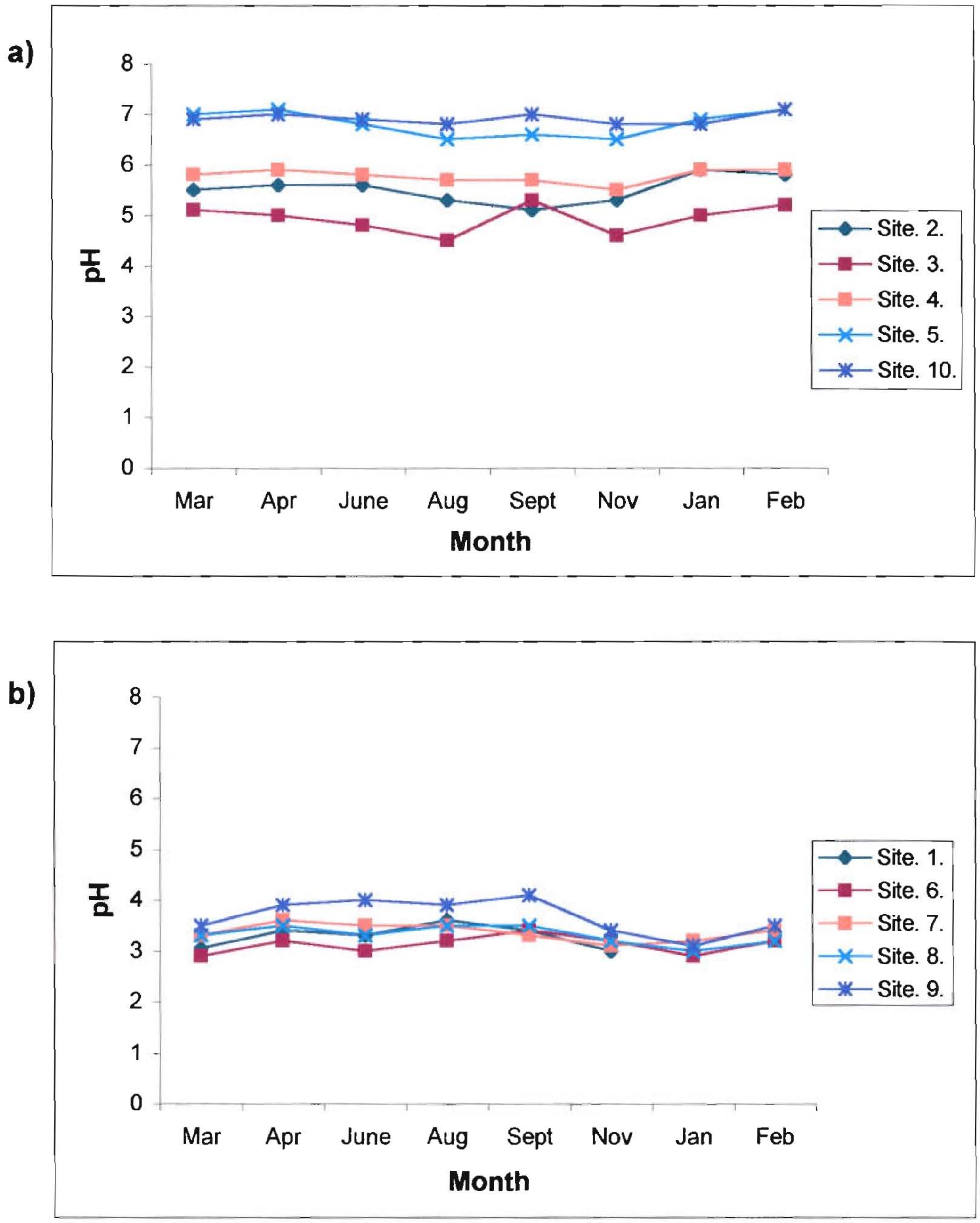


Fig 3.1 Stream water pH, March 1998 - February 1999.
a) Sites with pH > 4.5, **b)** Sites with pH < 4.5.

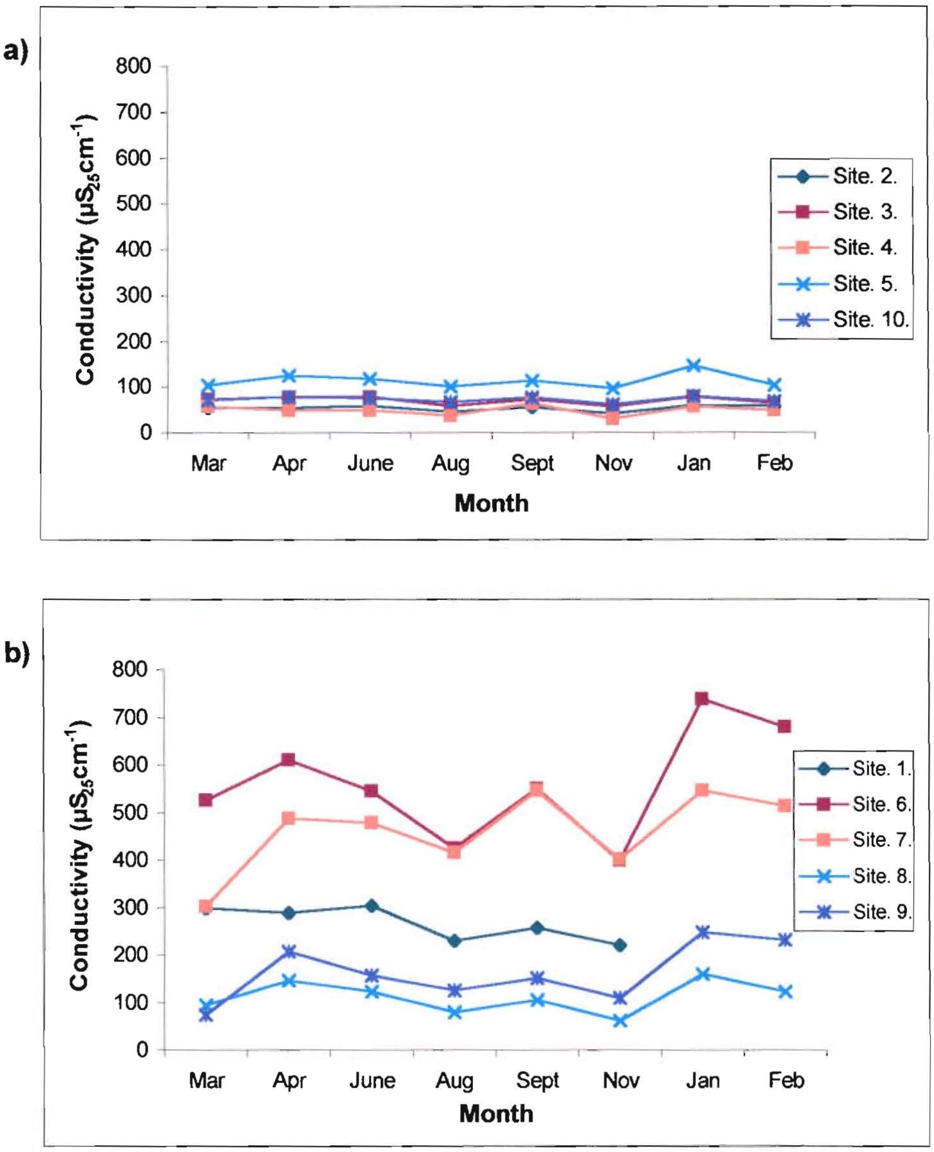


Fig 3.2 Stream water conductivity, March 1998 - February 1999.
a) Sites with pH > 4.5, **b)** Sites with pH < 4.5.

between control and acidic sites.

Sites 5 and 10 (control sites) had high mean alkalinity (46 mg L^{-1} and 30 mg L^{-1}) in comparison to acidic sites which displayed low or zero values (Table 3.2). Low alkalinity was correlated with low pH ($r_s = 0.967$, $P < 0.001$). Alkalinity was some what variable and no seasonal trend was evident (Fig 3.3a & b), however, a linear regression showed that alkalinity was significantly and positively related to discharge ($r^2 = 0.144$, $P < 0.05$). Differences between acidic and control sites were highly significant ($P < 0.001$).

Total dissolved aluminium was elevated at mine affected sites (0.02 to 5.83 mg L^{-1}), whereas at the control sites concentrations were < 0.06 mg L^{-1} (Table 3.2). No apparent seasonal trend was found, although values at most sites were marginally higher during March and decreased slightly heading into the cooler winter months (Fig 3.4a & b). A linear regression showed total dissolved aluminium to be negatively and significantly associated with discharge ($r^2 = 0.311$, $P < 0.05$), and is reflected in the decrease observed in winter (June – August) (Fig 3.4a & b). Differences in total dissolved aluminium between control sites and site 6 (acidic site) were highly significant ($P < 0.001$), however, no significant differences were observed between control sites and the other acidic sites.

Similarly, all mine-affected sites had elevated concentrations of total iron with mean values ranging from 0.2 to 2.35 mg L^{-1}. In contrast, both control sites had low concentrations of total iron with values ranging from 0 to 0.08 mg L^{-1} (Table 3.2). A slight seasonal pattern was apparent with most sites displaying high values during March followed by much lower values in June (Fig 3.5a & b) when water temperatures were cooler and discharge was greater. A linear regression showed total iron to be significantly and negatively associated with discharge ($r^2 = 0.186$, $P < 0.05$). Differences in total iron between control sites and Site 6 (affected by acid mine drainage) were highly significant ($P < 0.001$) but again no significance was observed between control sites and the other acidic sites.

Concentrations of nitrate-nitrogen did not vary much between sites and ranged from 0 (Sites 3, 5 and 8) to 0.1 mg L^{-1} (Sites 2, 5 and 10). There were no differences between mine affected and control sites (Table 3.2). Significant differences were observed between Site 8 and Sites 1 and 2 ($P < 0.05$). No seasonal pattern or stream water discharge trends were apparent.

Reactive phosphate concentrations also showed little variation among sites. Although mean values were slightly greater at the control sites (0.53 mg L^{-1} and 0.15 mg L^{-1}). No

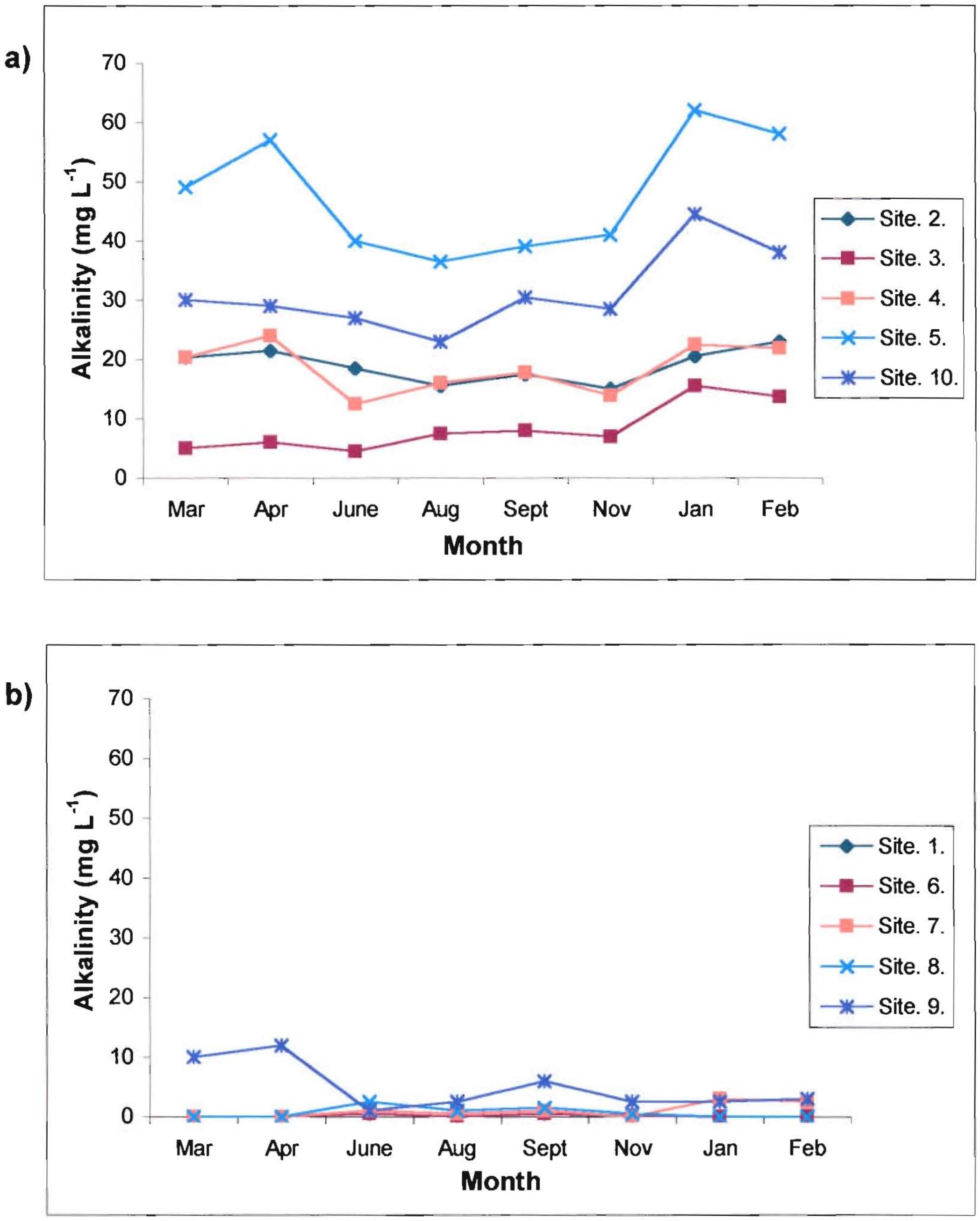
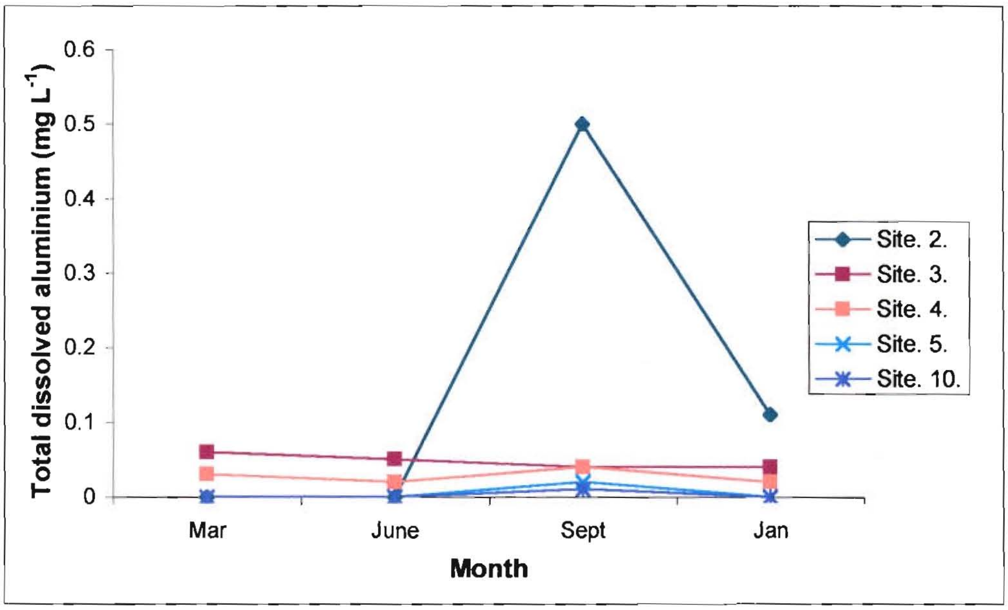


Fig 3.3 Stream water alkalinity, March 1998 - February 1999.
a) Sites with pH > 4.5, **b)** Sites with pH < 4.5.

a)



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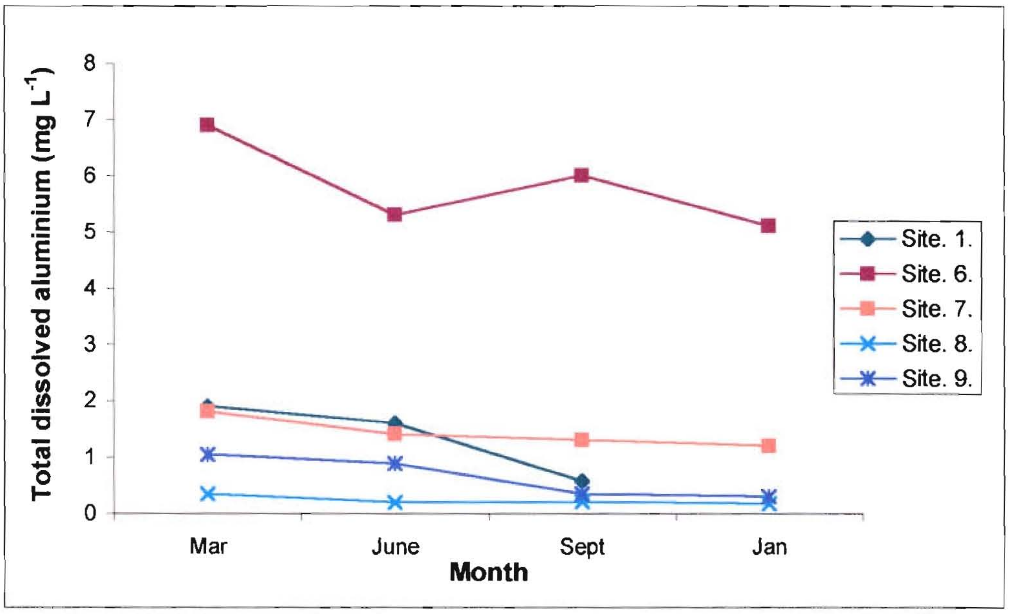
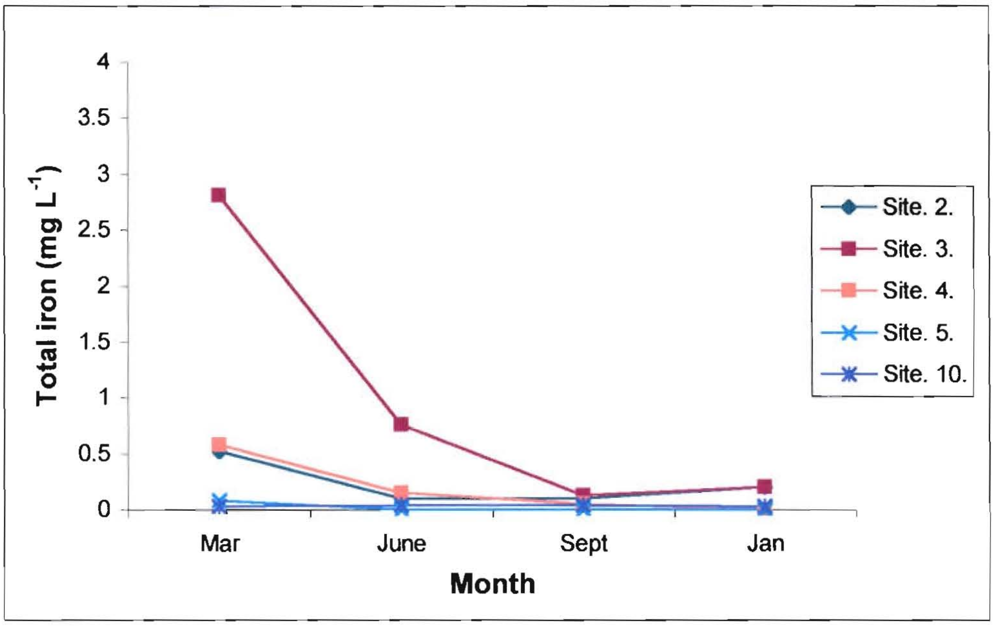


Fig 3.4 Stream water total dissolved aluminium, March 1998 - February 1999 **a)** Sites with pH > 4.5.
b) Sites with pH < 4.5. NB. scale difference.

a)



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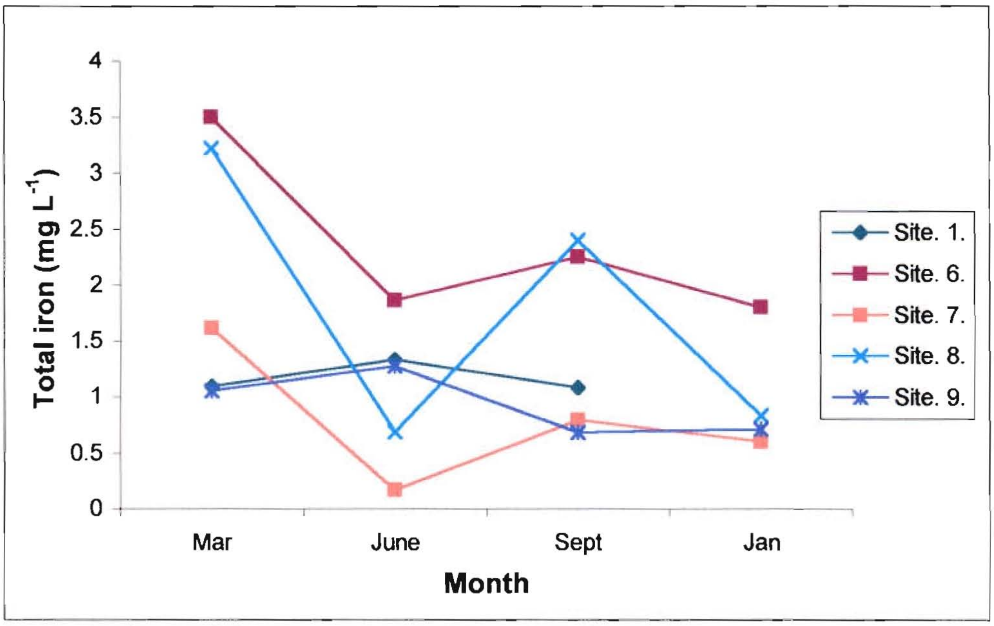


Fig 3.5 Stream water Total iron, March 1998 - February 1999.
a) Sites with pH > 4.5, **b)** Sites with pH < 4.5.

significant differences were observed between sites ($P > 0.05$). No seasonal trends were apparent, however, reactive phosphate concentrations decreased significantly with increasing discharge ($r^2 = 0.146$, $P < 0.05$).

Hyporheic stream waters

Hyporheic water chemical conditions measured at the 10 study sites between March 1998 and February 1999 are summarised in Table 3.3 and Figs 3.6 to 3.10.

Mean pH of hyporheic water ranged from 3.2 at Site 1 to 6.6 at Site 10 (control site). However, pH at all sites fluctuated within a range of 0.3 to 1.8 units during the study period. In general, pH was higher at all sites in the hyporheos than in surface water. The hyporheos, however, had a much greater range over which pH fluctuated during the study (Table 3.2 and Table 3.3). Differences in pH between acidic Site 1 and control sites were highly significant ($P < 0.001$), however, no other significant differences were observed between sites. Like surface water, pH did not display any clear seasonal trends although lowest readings for most sites were recorded during spring (September) (Fig 3.6a & b).

Conductivity ranged from 34 μS_{25} cm^{-1} to 430 μS_{25} cm^{-1}, and as in surface waters, conductivity was greatest at the most acidic site (Site 6) (Table 3.3). Conductivity values for hyporheic water followed the same low pH-high conductivity trend as found in surface water, however, overall mean conductivity values were lower in the hyporheic zone at most sites. Conductivity did not appear to display a seasonal pattern (Fig 3.7a & b). Strongly significant differences in conductivity ($P < 0.001$) were observed between Sites 4 and 6, and also between Site 6 and Sites 2, 3 and 4.

Sites 5 and 10 (control sites) had high mean alkalinities (38 mg L^{-1} and 33 mg L^{-1}, respectively) compared to acidic sites, which had means ranging from 0 to 12 mg L^{-1} (Table 3.3). Alkalinity values were fairly similar in surface and hyporheic waters although at most sites they were slightly lower in the hyporheic zone. No clear seasonal trends were apparent, although at most sites the lowest readings were obtained in September (Fig 3.8a & b). Differences in alkalinity between Sites 1 and 7 and the control sites were highly significant ($P < 0.001$).

Total dissolved aluminium concentrations were elevated at mine affected sites, with means ranging from 0.02 mg L^{-1} (Site 2) to 4.65 mg L^{-1} (Site 6) (Table 3.3). In contrast, aluminium could not be detected at the two control sites. Concentrations of total dissolved aluminium were similar in both surface and hyporheic waters. No seasonal trend in

Table 3.3 Hyporheic water chemistry at the ten study sites (mean values with ranges in parentheses).

Site	pH	Conductivity ($\mu S_{25} cm^{-1}$)	Alkalinity ($mg\ L^{-1}$)	Nitrate - Nitrogen ($mg\ L^{-1}$)	Reactive Phosphate ($mg\ L^{-1}$)	Total Iron ($mg\ L^{-1}$)	Total dissolved Aluminum ($mg\ L^{-1}$)
Site 1	3.2 (3.1 - 3.4)	247 (233 - 259)	0	0.01 (0 -0.02)	0.01 (0 - 0.04)	4.2 (0.67 - 9.6)	2.3 (2.1 - 2.4)
Site 2	6 (5.2 - 6.5)	46 (40 - 51)	12 (11 - 14)	0.01 (0.01 - 0.02)	0.01 (0 - 0.02)	0.3 (0.1 - 0.5)	0.02 (0 - 0.05)
Site 3	6.2 (5 - 6.8)	54 (47 - 69)	5 (4.5 - 5.5)	0.03 (0.01 - 0.04)	0.07 (0.01 - 0.02)	0.7 (0.19 - 1.1)	0.03 (0.01 - 0.05)
Site 4	5.9 (5.5 - 6.1)	61 (34 - 85)	12 (8 - 18)	0.03 (0 - 0.05)	0.14 (0 - 0.5)	0.36 (0.14 - 0.69)	0.003 (0 - 0.01)
Site 5	6.5 (5.8 - 6.9)	113 (93 - 133)	38 (30 - 45)	0.05 (0 - 0.08)	0.5 (0.05 - 1.51)	0.07 (0.03 - 0.1)	0
Site 6	3.4 (2.8 - 3.9)	412 (383 - 430)	2 (1 - 3.5)	0.012 (0.01 - 0.02)	0.02 (0 - 0.04)	8.3 (6 - 10.5)	4.56 (4.05 - 5.1)
Site 7	3.3 (3.1 - 3.5)	410 (393 - 423)	0.3 (0 - 0.5)	0.012 (0 - 0.02)	0.02 (0 - 0.05)	0.87 (0.63 - 1.28)	1.62 (1.43 - 1.8)
Site 8	3.5 (3.3 - 3.6)	108 (93 - 145)	1.5 (0.5 - 2.5)	0.02 (0 - 0.03)	0.04 (0 - 0.05)	0.6 (0.33 - 0.91)	0.4 (0.3 - 0.7)
Site 9	4 (3.9 - 4.1)	113 (80 - 151)	4 (3.5 - 5)	0.01 (0 - 0.02)	0.01 (0 - 0.04)	1.7 (1.19 - 2.19)	0.1 (0.05 - 0.19)
Site 10	6.6 (6 - 6.9)	76 (69 - 82)	33 (28 - 37)	0.048 (0.04 - 0.05)	0.08 (0.02 - 0.16)	0.6 (0.19 - 0.89)	0

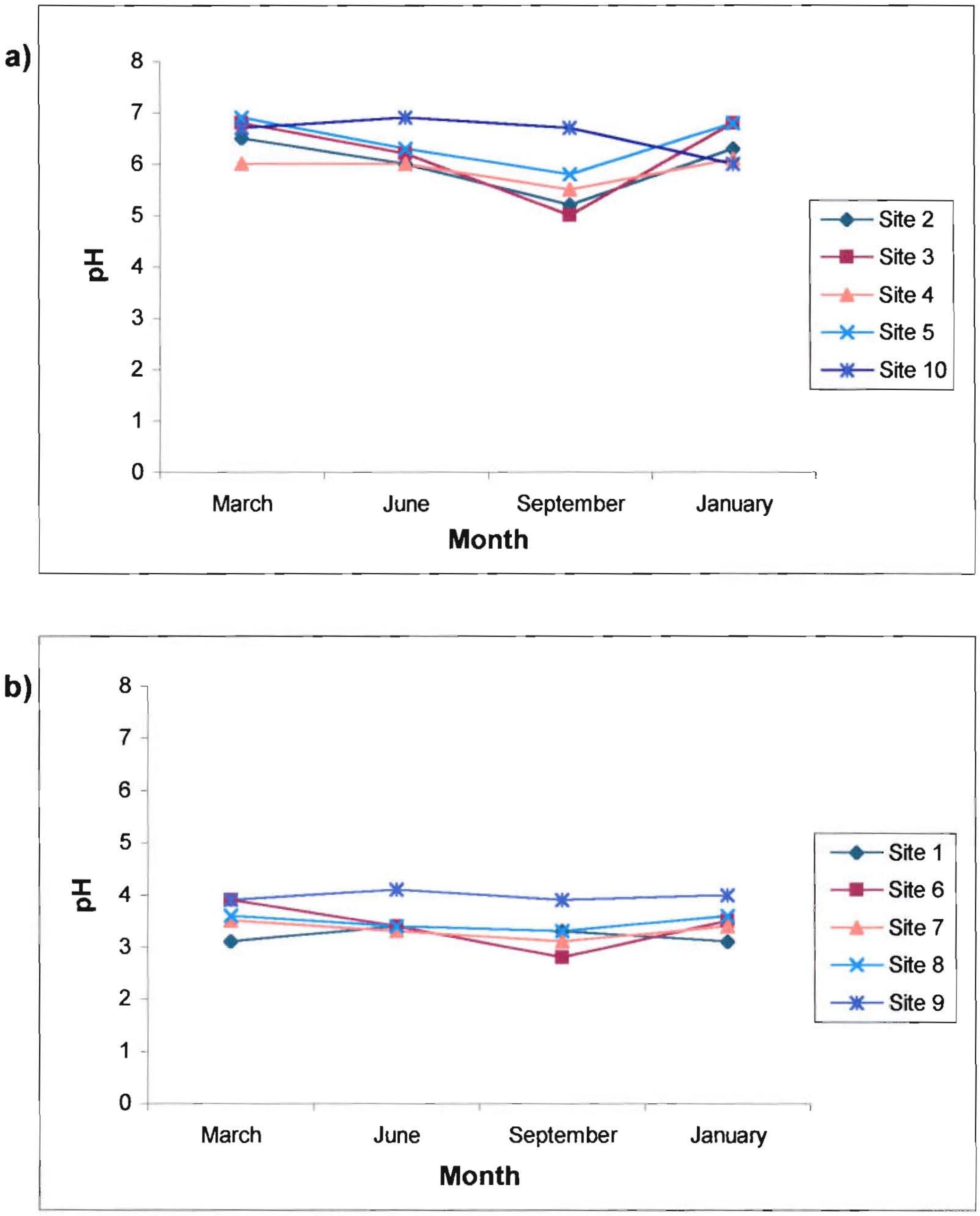
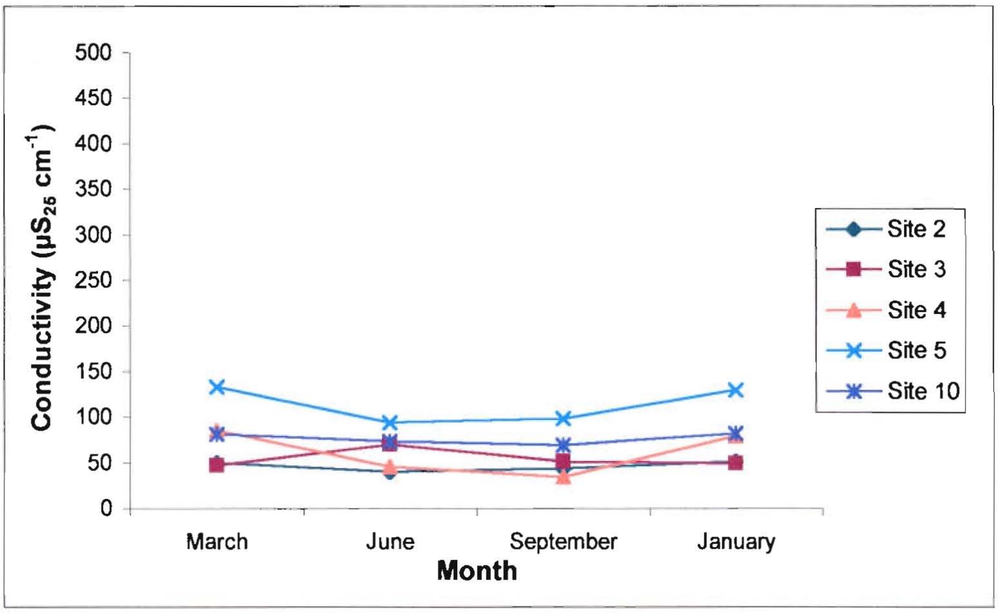


Fig 3.6 Hyporheic water pH, March 1998 - January 1999.
a) Sites with pH > 5.0, **b)** Sites with pH < 5.0.

a)



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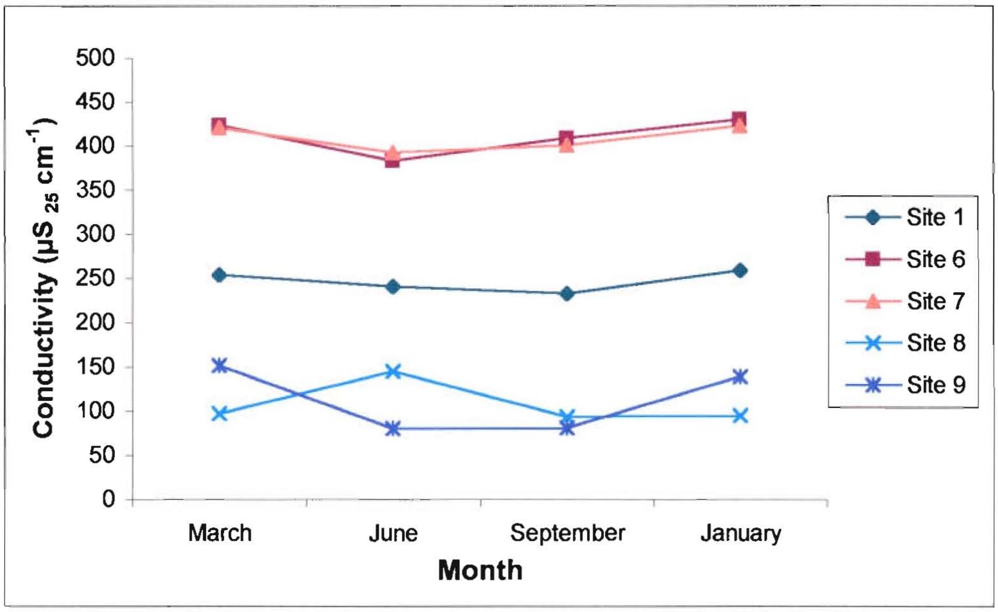
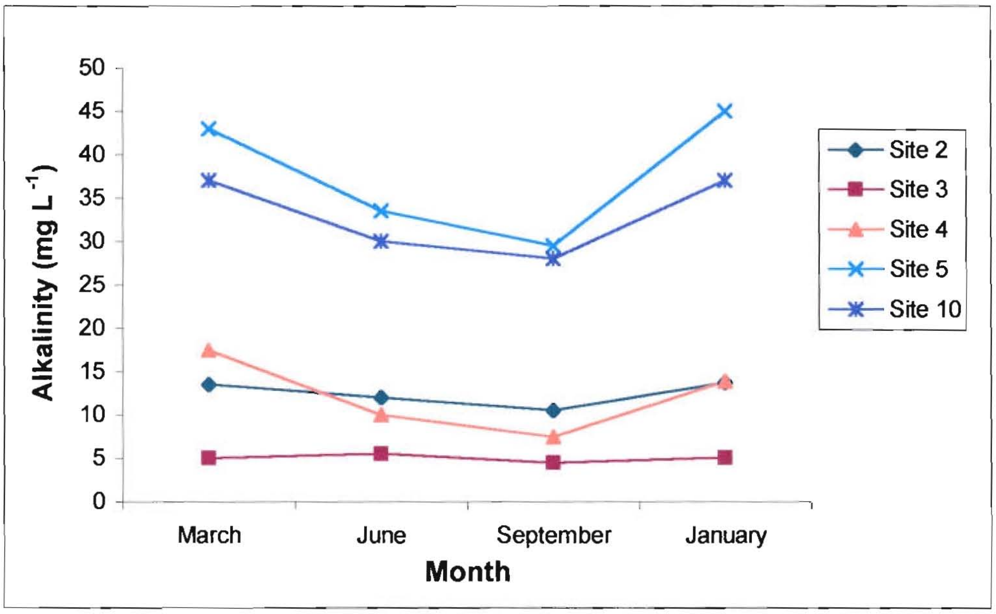


Fig 3.7 Hyporheic water conductivity, March 1998 - February 1999.
a) Sites with pH > 5.0, **b)** Sites with pH < 5.0.

a)



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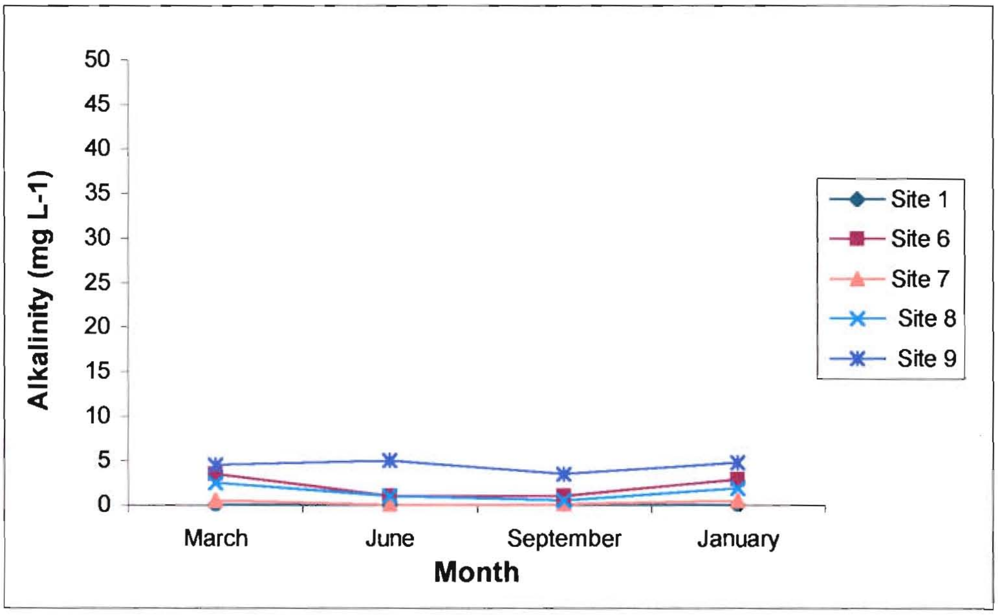


Fig 3.8 Hyporheic water alkalinity, March 1998 - January 1999.
a) Sites with pH > 5.0, **b)** Sites with pH < 5.0.

aluminium concentration was apparent although like surface water, most sites had slightly higher values in March than in June (winter) (Fig 3.9a & b). Differences in total dissolved aluminium between control sites (Sites 5 and 10) and Sites 1 and 6, and also between Sites 4, 5 and 10 and Sites 1 and 6 were highly significant ($P < 0.001$).

All mine affected sites also had elevated concentrations of total iron with mean values ranging from 0.3 mg L^{-1} to 8.3 mg L^{-1} (Table 3.3). Site 5 (control) had the lowest total iron reading of 0.07 mg L^{-1}. Apart from higher mean iron concentrations recorded at Site 1 (4.2 mg L^{-1}) and Site 6 (8.3 mg L^{-1}) all other concentrations were similar to those found in surface water (Table 3.2). No seasonal trend in iron concentration was apparent (Fig 3.10a & b). Differences in total iron between Sites 2 and 5 and Site 6, and also between Site 5 (control) and Sites 1, 6 and 9 were highly significant ($P < 0.001$).

Nitrate-nitrogen levels varied little between sites with means ranging from 0.01 mg L^{-1} to 0.05 mg L^{-1} (Table 3.3). Significant differences were found among sites ($P < 0.05$), however, nitrate-nitrogen concentrations recorded from hyporheic waters were similar to those found in surface water at the same sites (Table 3.2).

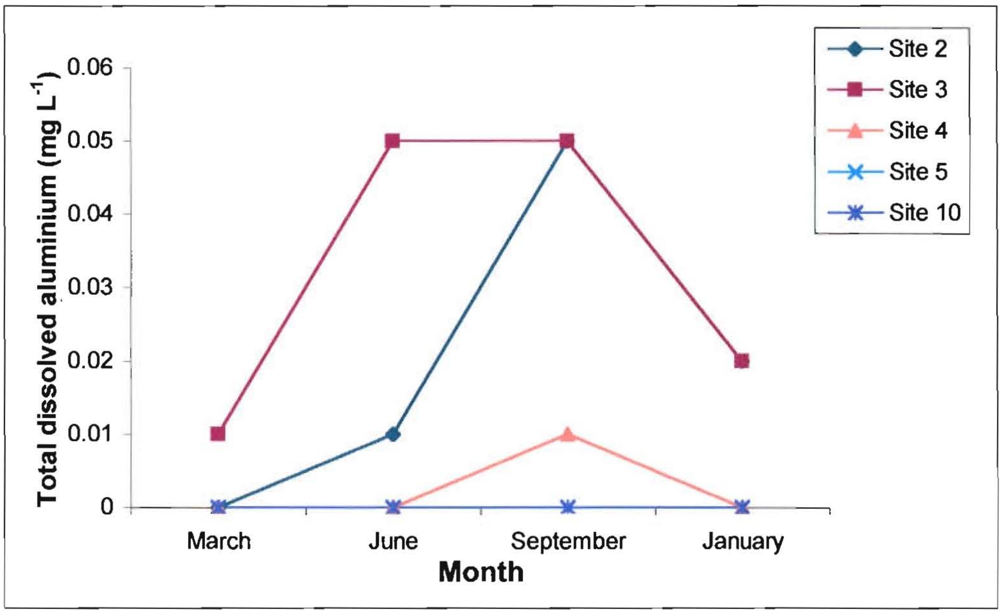
Site means for reactive phosphate ranged from 0.01mg L^{-1} at 3 acidic sites (Sites 1, 2 and 9) to 0.5 mg L^{-1} at Site 5 (control site). Reactive phosphate concentrations in the hyporheos were similar to those found in surface waters, with the highest reading in both cases being for Site 5. Concentrations of reactive phosphate were significantly different among sites ($P < 0.05$).

Relationships among measured physico-chemical variables

Five of the seven chemical variables measured in surface water were strongly intercorrelated ($r_s = 0.721 - 0.967$; $P < 0.05$) (Table 3.4). However nitrate-nitrogen and reactive phosphate were not correlated with other chemical variables. With the exception of nitrate-nitrogen and reactive phosphate, they were also strongly correlated with distance from the nearest mine. With the exception of nitrate-nitrogen, all chemical variables were strongly correlated with stream discharge ($r_s = 0.574 - 0.919$; $P < 0.05$) (Table 3.4). Chemical variables were also strongly intercorrelated in hyporheic water samples ($r_s = 0.571 - 0.940$; $P < 0.05$) and both nitrate-nitrogen and reactive phosphate showed stronger correlations with other variables than they did in surface water (Table 3.5). Distance from the nearest mine was significantly correlated with water chemical variables ($P < 0.05$) as in surface waters.

Five of the seven surface water chemical variables measured were strongly

a)



b)

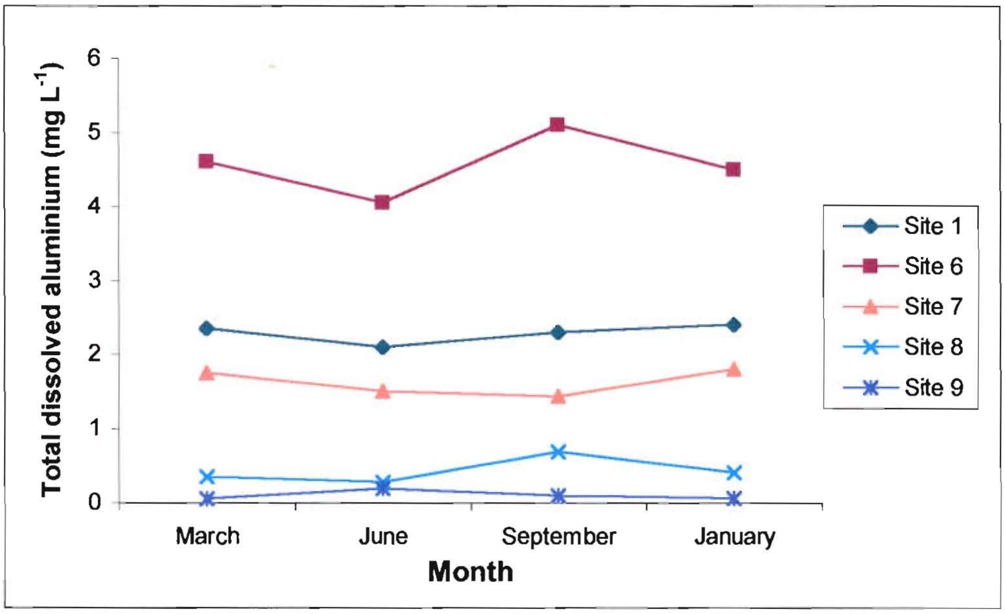


Fig 3.9 Hyporheic water Total dissolved Aluminium, March 1998 - January 1999, **a)** Sites with pH > 5.0. **b)** Sites with pH < 5.0. N.B. Scale difference

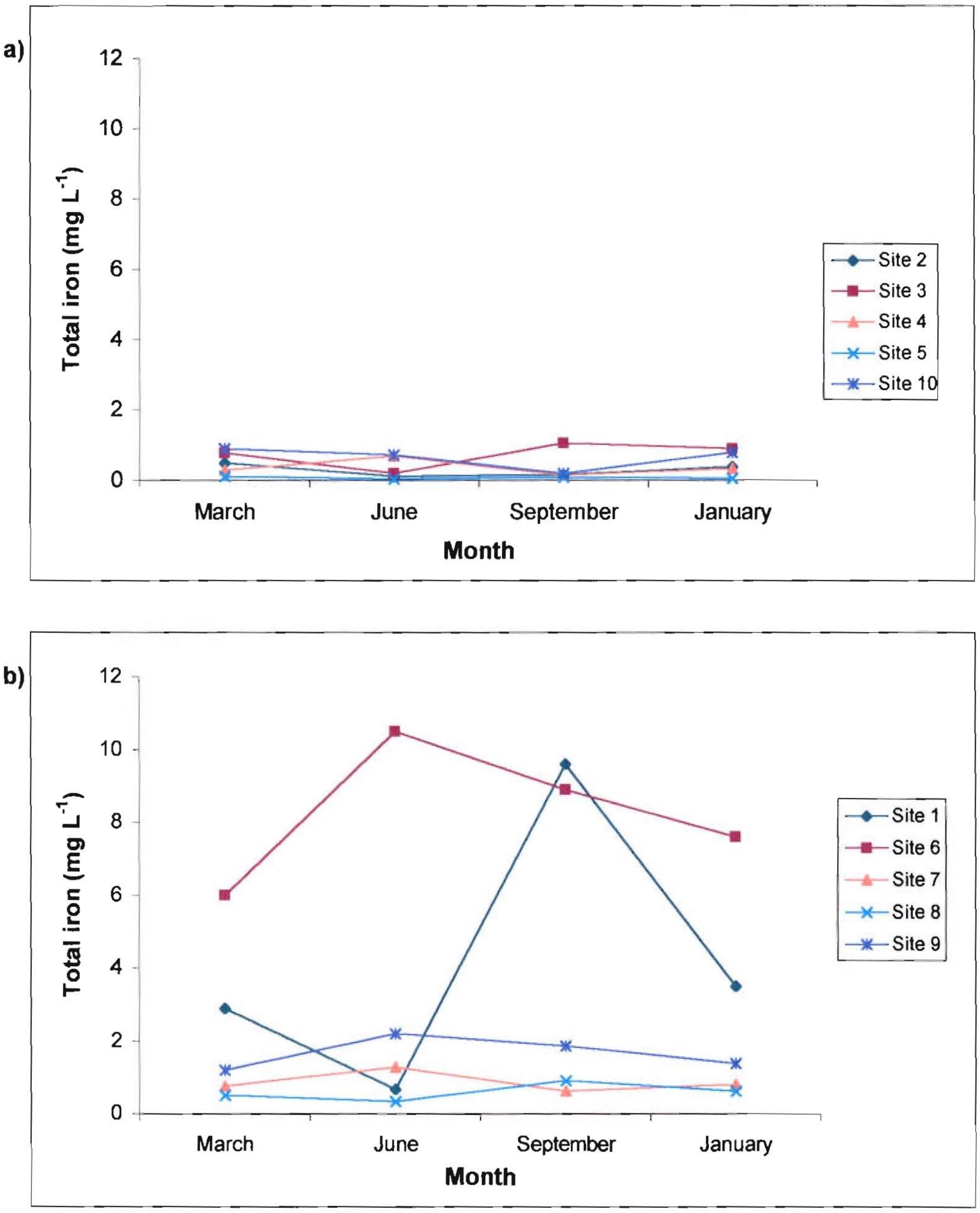


Fig 3.10 Hyporheic water Total iron, March 1998 - January 1999.
a) Sites with pH > 5.0, **b)** Sites with pH < 5.0.

Table 3.4 Correlations (r_s) among physico-chemical factors (mean values) measured for surface water at the 10 sites.
*, P < 0.05; **, P < 0.01; ***, P < 0.001

	pH	Conductivity	Alkalinity	Aluminium	Iron	Nitrate-Nitrogen	Phosphate	Distance
Conductivity	0.809**	-						
Alkalinity	0.967***	0.842**	-					
Aluminium	0.918***	0.867**	0.891***	-				
Iron	0.936***	0.721*	0.915***	0.794**	-			
Nitrate-Nitrogen	0.118	0.006	0.025	0.124	0.222	-		
Phosphate	0.235	0.092	0.265	0.277	0.375	0.11	-	
Distance	0.853**	0.854**	0.866**	0.890***	0.731*	0.186	0.384	-
Discharge	0.919***	0.882***	0.891***	0.884***	0.589*	0.191	0.574*	-

Table 3.5 Correlations (r_s) among physico-chemical factors (mean values) measured for hyporheic water at the 10 sites.
*, P < 0.05; **, P < 0.01; ***, P < 0.001

	pH	Conductivity	Alkalinity	Aluminium	Iron	Nitrate-Nitrogen	Phosphate	Distance
Conductivity	0.663*	-						
Alkalinity	0.939***	0.571*	-					
Aluminium	0.912***	0.662*	0.912***	-				
Iron	0.626*	0.604*	0.699*	0.759**	-			
Nitrate-Nitrogen	0.679*	0.217	0.605*	0.656*	0.563	-		
Phosphate	0.616*	0.222	0.585*	0.648*	0.599*	0.978***	-	
Distance	0.854**	0.578*	0.787**	0.924***	0.716*	0.817**	0.808**	-

intercorrelated with the corresponding hyporheic water variables (r_s = 0.571 – 0.976; $P < 0.05$). However, surface water nitrate-nitrogen and reactive phosphate were not correlated with those in hyporheic water.

Epilithon

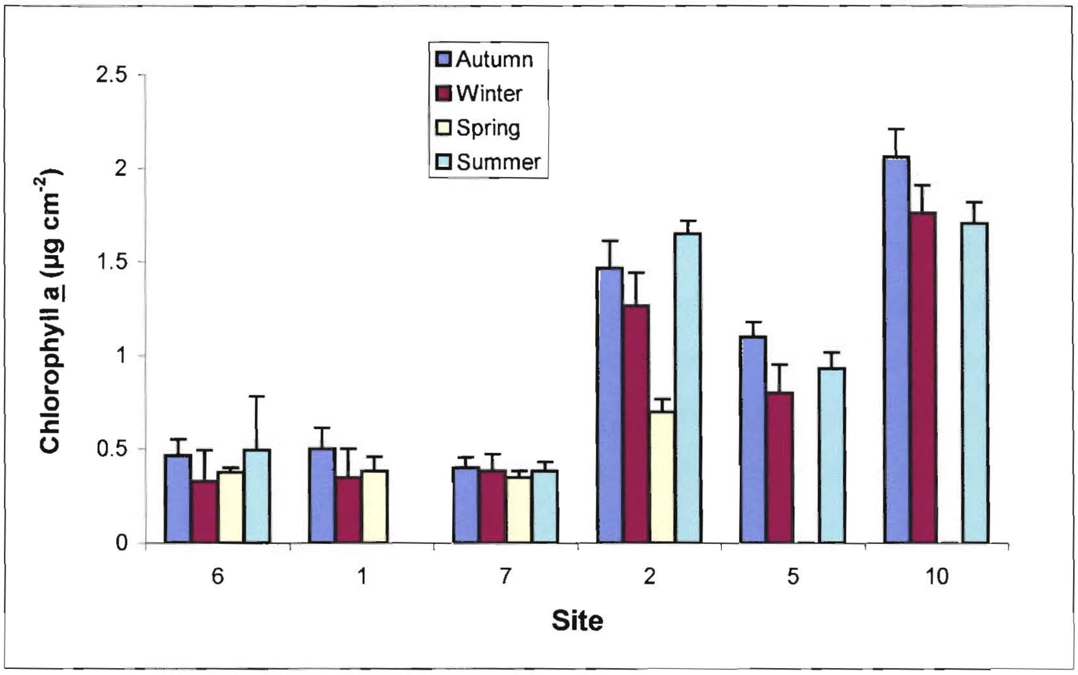
The six sites chosen for epilithic colonisation experiments (Sites 1, 2, 5, 6, 7, 10) differed in pH and associated chemical variables. Sites 5 and 10 were control sites of near neutral pH (mean values; 6.8 and 6.9, respectively) (Table 3.2). The other four sites were affected to varying degrees by acid mine drainage and had pH means ranging from 3.2 to 5.7 (Table 3.2). Total iron means ranged from 0.02 mg L^{-1} at the control site (Site 5) to 2.35 mg L^{-1} at the most acidic site (Table 3.2). Concentrations of total dissolved aluminium followed a similar trend with lowest concentrations observed at control sites and highest concentrations at acidic sites (Table 3.2). Concentrations of nitrate-nitrogen did not differ among sites, however, phosphate was slightly higher at the control sites (Table 3.2).

No tiles were collected from Sites 5 or 10 in spring because heavy rainfall and subsequent high flows washed the tiles away. Tiles were not placed at Site 1 during summer because the stream dried up completely in January and February 1999.

Temporal changes in algal biomass and rates of community respiration are shown in Fig 3.11a & b. Mean chlorophyll a concentrations ranged from 0.33 μg cm^{-2} to 2.06 μg cm^{-2} (Fig 3.11a) and were greatest at all sites, except Site 6, in autumn. The lowest and highest chlorophyll a concentrations were recorded at the most acidic site (Site 6) and the most neutral site (Site 10), respectively. Chlorophyll a values were lower at the more acidic sites in all seasons. A two-way Analysis of Variance showed that the differences in chlorophyll a concentrations were highly significant among both sites and seasons ($P < 0.001$). Tukey tests showed that Sites 2, 5 and 10 differed significantly from each other and that chlorophyll a concentrations at all three were significantly different from those at Sites 1, 6 and 7, which did not differ from each other. They also indicated that chlorophyll a concentrations did not differ in autumn, winter and summer, but were significantly lower in spring.

Mean community respiration rates ranged from 0.07 μg O_2 cm^{-2} hr^{-1} to 1.98 μg O_2 cm^{-2} hr^{-1} (Fig 3.11b) and were greatest at all sites in autumn. Community respiration differed significantly among sites ($P < 0.05$) with respiration at Sites 2 and 7 being significantly lower than at Site 1. Unlike chlorophyll a, mean community respiration was not correlated with site

a)



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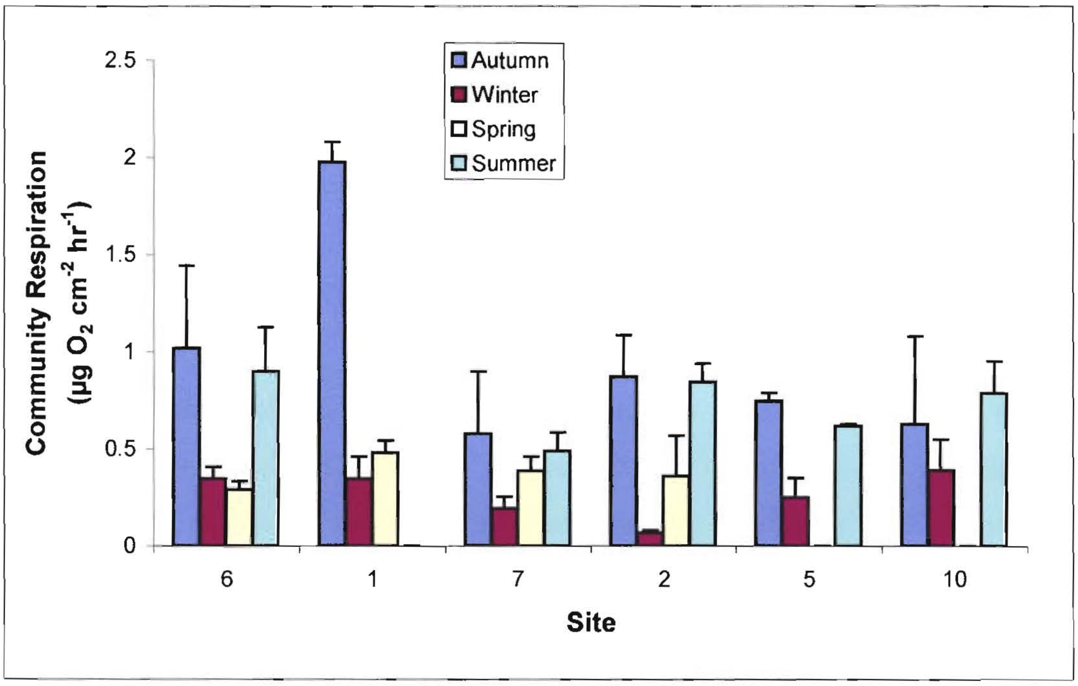


Fig 3.11 a) Mean Algal Biomass (± 1SE) on tiles (as chlorophyll a) in six streams and four seasons.
b) Mean Community Respiration (± 1SE) measured on tiles in the six streams and four seasons.
(Sites arranged from most acidic to least, left to right).

pH (P = 0.018). Rates of community respiration were strongly affected by season (P < 0.001), with rates in winter and spring differing from one another and from those obtained in autumn and summer (Tukey tests). Community respiration was not significantly different (P > 0.05) in autumn and summer.

Relationships between chlorophyll a and community respiration in each season are shown in Fig 3.12. At no time during the study were chlorophyll a and community respiration correlated with each other (r_s > 0.05) (Fig 3.12). In some studies of acid streams, higher epilithic algal biomass was found at low pH (Hendrey, 1976; Hall *et al.*, 1980; Mulholland *et al.*, 1986), but this was not the case in my study. Instead, my findings were similar to those of Collier (1988) who found that algal biomass was greater on stones in circumneutral pH streams than at low pH brownwater sites with similar degrees of streambed shading. However, experiments in artificial channels suggested that low pH was not the factor restricting algal biomass at the brownwater sites (Collier, 1988), and indicated that other factors such as reduced light intensity or low nutrient levels may have been limiting. In my study no relationships between nitrate and phosphate concentrations in stream water and algal biomass were recorded (Fig 3.13a & b), but experimental studies would be necessary to confirm whether either nutrient was limiting.

SUMMARY

Ten streams, eight affected by coal mine drainage to varying degrees, and two acting as controls were compared with respect to chemical (pH, conductivity, alkalinity, total dissolved aluminium, total iron, nitrate-nitrogen and reactive phosphate) and physical factors (stream width, depth, discharge, temperature, total stability, bottom stability and distance from the nearest mine).

Although all streams were small (mean widths 0.41 to 2.12 m) their physical characteristics varied among them and between seasons. In general, mine affected sites had higher temperatures than control sites. During periods of high flow, water temperatures declined and the range of temperatures between sites was also reduced. These results are consistent with those obtained by Graesser (1988) in mildly acid, brown water streams in South Westland. Depth and discharge varied over narrow ranges among sites, however, and the differences are unlikely to be biologically significant as most of the common invertebrates found in New Zealand streams occur over a wide range of stream sizes and discharges

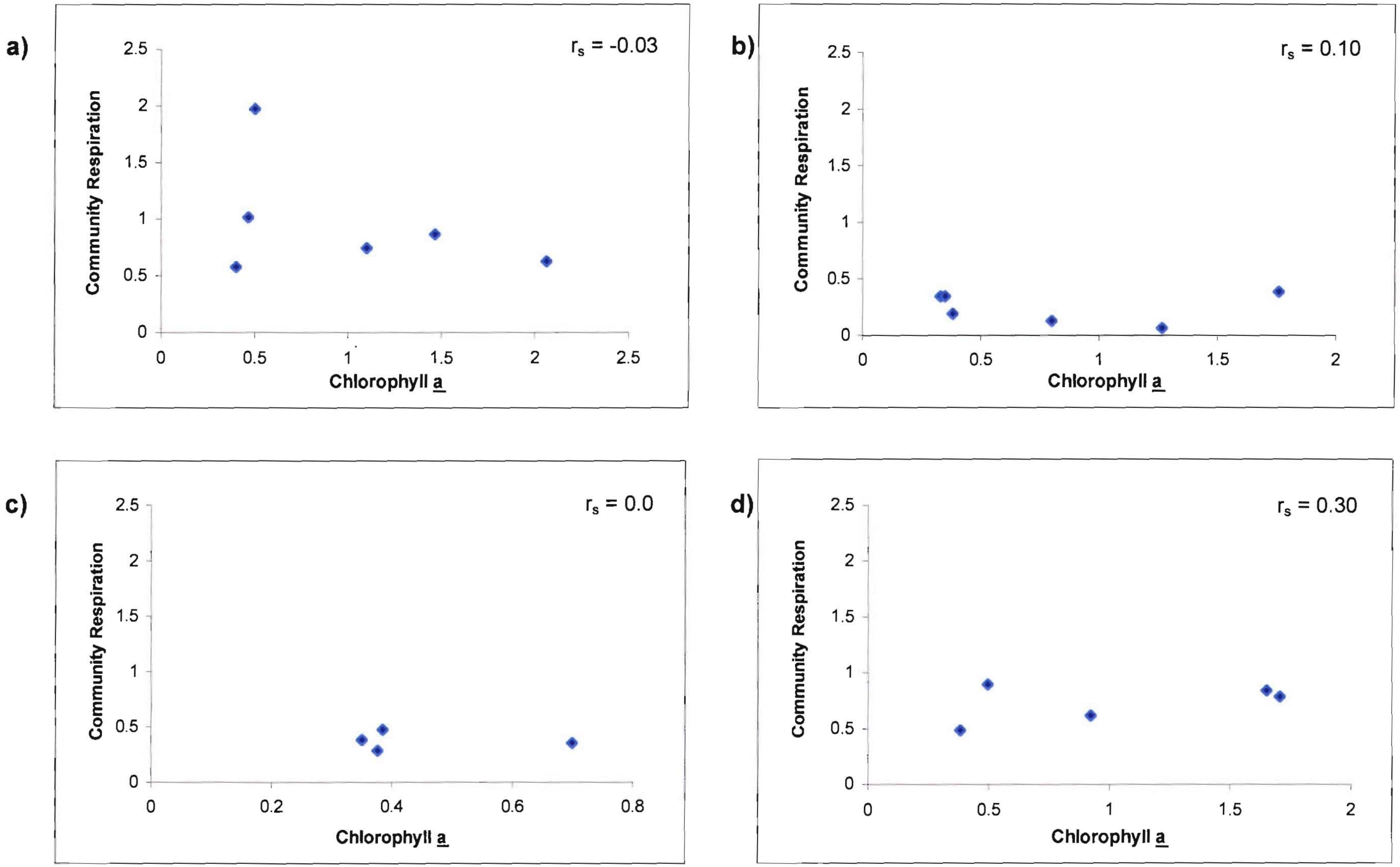
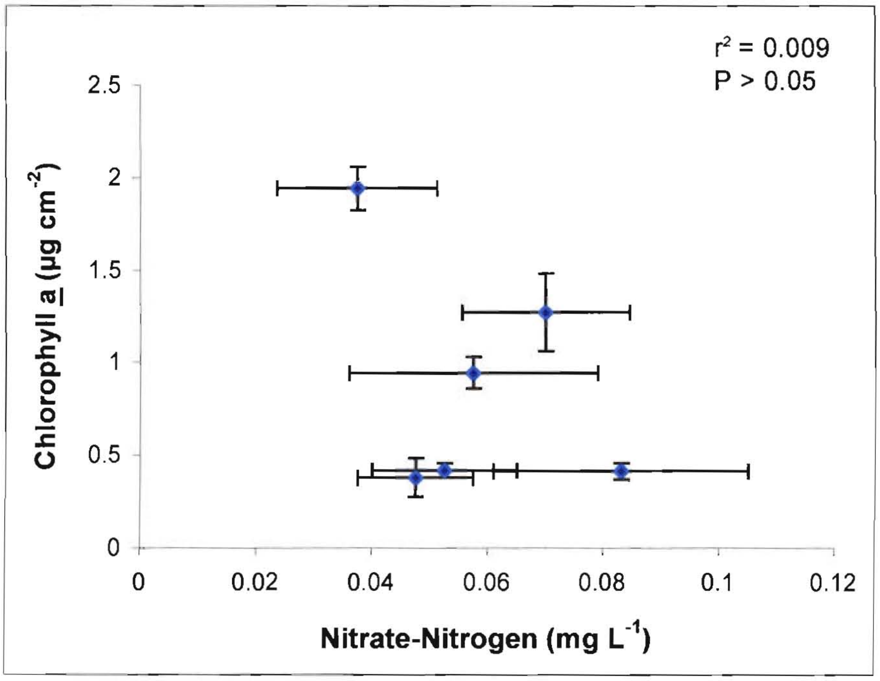


Fig 3.12 Correlations between Community Respiration (µg O_2 cm^{-2} hr^{-1}) and Chlorophyll a (µg cm^{-2}), **a)** Autumn, **b)** Winter, **c)** Spring, **d)** Summer, **d)** Summer (rs = Spearman's rank coefficient: all 4 vlaues shown are not signifcant, P > 0.05).

a)



b)

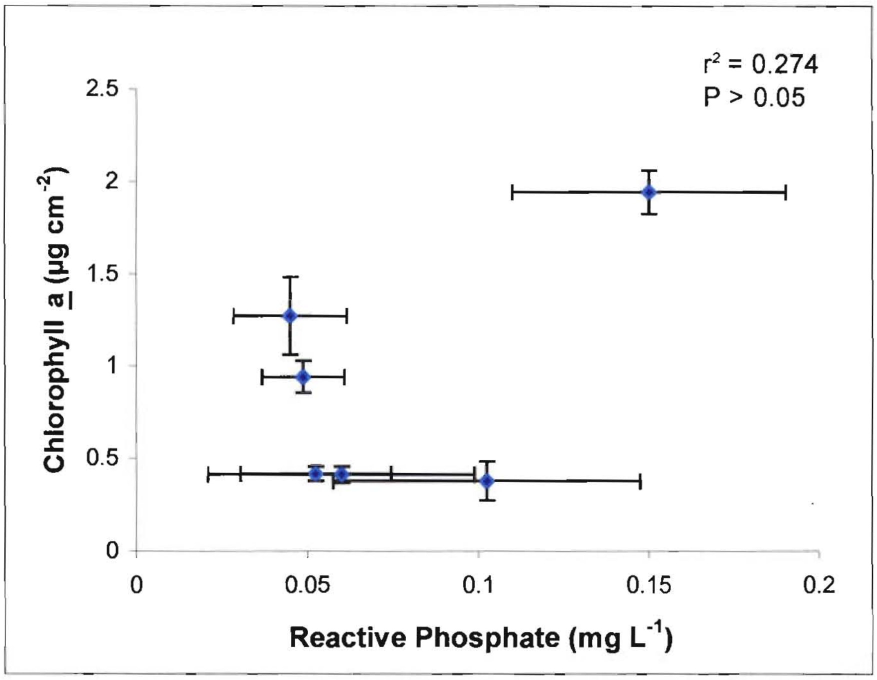


Fig 3.13 a) Mean algal biomass (± 1SE) on tiles (as chlorophyll a) in four seasons versus mean nitrate-nitrogen (± 1SE) content in the stream in the four seasons (six sites).
b) Mean algal biomass (± 1SE) on tiles (as chlorophyll a) in four seasons versus mean reactive phosphate (± 1SE) content in the stream in four seasons (six sites).

(Collier, 1994; Quinn & Hickey, 1990). Many of the sites also had physically unstable beds and banks as indicated by channel stability scores (Pfankuch, 1975), with 7 of the 10 streams having a fair rating on a scale of excellent, good, fair and poor.

Results of my water chemistry analyses indicate that conditions in the surface waters examined conformed generally to those reported by, Dills & Rogers (1974), Letterman & Mitsch (1978), Winterbourn & McDiffett (1996) and Winterbourn (1998). Thus acid mine drainage-affected streams were characterised by low pH, little or no measurable alkalinity and high conductivity reflecting high concentrations of metal ions. pH ranged from 2.9 to 7.1, whereas, Penny (1987) recorded relatively high pH readings (5.8 to 7.8) in a study of streams in three catchments polluted by gold mining effluents on the Coromandel Peninsula, New Zealand. The differences reflect the different geology's of the areas and the nature of mining undertaken historically in the two regions.

Higher total dissolved aluminium and total iron concentrations were also observed in my study as pH declined, with values ranging from 0 to 6.9 mg L^{-1} and 0 to 3.5 mg L^{-1}, respectively. Elevated concentrations of both aluminium and iron were recorded by Koryak, Sharpiro & Sykora (1972) (iron only), Hargreaves, Lloyd & Whitton (1975), Greenfield & Ireland (1978) (iron only), Engelman & McDiffett (1996), Winterbourn & McDiffett (1996) and Winterbourn (1998) in streams affected by acid mine drainage, and by Hall *et al.* (1980) in experimentally acidified streams in New Hampshire, U.S.A. Levels of both nitrate-nitrogen and reactive phosphate were low or undetectable in the present study and are consistent with results obtained by Dills & Rogers (1974) who found that nitrate and phosphate were reduced by 83 % and 60 % respectively compared with a control site at coal mine drainage-affected sites. However, Hargreaves *et al.* (1975) observed high concentrations of phosphate in streams affected by acid mine drainage, again showing the importance of local conditions on water chemistry. I also found that stream water chemistry variables intensified at low discharge, consistent with findings of Herricks (1977) and Hall et al. (1980). In contradiction to these results, Parsons (1968) and Scullion & Edwards (1980) noted an increase in effluent ions with increased stream flow, inferring that ion concentrations in runoff were high and therefore did not have a diluting effect.

In addition to surface waters, mine drainage can be expected to contaminate the hyporheic zone where ground and surface waters mix. In my study, the water chemistry of hyporheic samples was very similar to that found in surface water, although conductivity and alkalinity were generally slightly lower and pH slightly higher. Comparisons of highly acidic sites (pH < 4.5) and sites with pH > 4.5 within the hyporheos, indicated that conductivity, total dissolved aluminium and total iron were slightly elevated at acidic sites and pH was slightly lower. These findings parallel those of Gray (1996) who found that pH was lower, conductivity higher and concentrations of total iron were higher in the hyporheos of streams near the Avoca mines in County Wicklow, Ireland. In my study, concentrations of total dissolved aluminium, total iron, nitrate-nitrogen and reactive phosphate were relatively similar in both surface and hyporheic waters. However, Nelson et al. (1993) found that relative to surface water, hyporheic waters had higher concentrations of metal ions.

The epilithon present on the bed of a stream can also be adversely affected by acid mine drainage, and in turn it might be expected to influence density and production of benthic invertebrates. Madsen (1974) suggested that the organic based film adhering to stones in streams (the organic layer) is an important food source for stonefly larvae in Denmark, and Winterbourn (1976) proposed that organic layers were possibly the major feeding sites of benthic invertebrates in New Zealand forest streams.

My research showed that algal biomass (measured as chlorophyll a) was relatively low at all sites, and decreased with decreasing pH and elevated aluminium and iron concentrations. Similar results were obtained by McKnight & Feder (1984) and Davis-Colley *et al.* (1992), and also by Maurice et al. (1986) in artificial stream studies undertaken in the Upper Peninsula of Michigan. However, greater algal accumulation was reported to occur in artificially acidified streams by Hendrey (1976). Hall *et al.* (1980) also observed increased amounts of periphyton chlorophyll in an experimentally acidified stream in the Hubbard Brook Experimental Forest, New Hampshire, although differences in chlorophyll a concentration between acidified and non acidified reaches was eliminated following a storm event.

On the West Coast of the South Island of New Zealand, where the present study was conducted, spates and small floods occur frequently. The abrasive action of high flows may therefore limit epilithic algal biomass on stones as found by Moss (1968). This factor coupled with low nitrate-nitrogen and reactive phosphate concentrations in the streams selected for in this study could explain the low algal biomass at acidic sites.

Chapter Four

INVERTEBRATE FAUNA

INTRODUCTION

Relationships between biotic and abiotic factors and the composition and structure of lotic macroinvertebrate communities have received considerable attention in recent years. Stream water chemistry (Okland & Okland, 1986; Sutcliffe & Hildrew, 1989; Howells, 1995), predation (Allan, 1983), competition (Hart, 1983) and stream water flow (Eddington, 1968) have all been implicated in various studies.

Studies of streams affected by acid precipitation in the Northern Hemisphere have shown that low pH is a primary factor limiting stream invertebrate distribution (e.g., Allard & Moreau, 1987; Weatherley & Ormerod, 1987; Wade *et al.*, 1989). Several studies carried out on streams affected by acid mine drainage, both in New Zealand (e.g., Winterbourn & McDiffett, 1996; Winterbourn, 1998) and overseas (e.g., Dills & Roger, 1974; Hargreaves, Lloyd & Whitton, 1975; Tomkiewicz & Dunson, 1977; Scullion & Edwards, 1980; Rasmussen & Lindgaard, 1988) have found strong relationships between the chemical nature of stream water and distributions of benthic invertebrates.

Low pH (pH < 5.5), Northern Hemisphere waters typically have lower species richness and abundance than comparable circumneutral environments (Otto & Svensson, 1983; Rosemond *et al.*, 1992). A decline in abundance of Ephemeroptera (mayfly) taxa has frequently been recorded at pH 5.4 – 5.7 (Feldman & Connor, 1992). In the River Duddon and its tributaries where faunas were dominated by stoneflies, Sutcliffe & Carrick (1973) found that mayflies and many caddisfly species were absent at pH < 5.7. In a small mountain stream in western Pennsylvania affected by coal mine drainage, Letterman & Mitsch (1978) found about a quarter as many species in streams with pH < 6 and Fe > 3.6 mg L^{-1} than in less acidic streams (pH > 6, Fe < 0.2 mg L^{-1}). Trichoptera, Ephemeroptera and Diptera dominated the control streams, whereas Chironomidae (Diptera) were most tolerant of mine drainage, although only occurring in low densities. Ephemeropterans were totally absent from one of the three acidic streams. Greenfield & Ireland (1978) reported that Plecoptera, Ephemeroptera and Trichoptera were absent from sites affected by coalmine spoils in the

Burnley area of Lancashire. The most common macroinvertebrate species found in polluted waters were Oligochaeta and the larvae of ceratopogonid and chironomid midges.

In contrast, Winterbourn and Collier (1987) found that the mayfly *Deleatidium* was the most abundant insect in many naturally acidic brown water streams in New Zealand. Subsequently, a small number of studies of West Coast streams affected by acid mine drainage, reported low diversities of aquatic invertebrates (including species of Ephemeroptera, Plecoptera, Trichoptera and Diptera) characteristic of "clean", stony streams down to a pH of about 4 (Winterbourn & McDiffett, 1996; Winterbourn, 1998).

Experimental studies have also indicated that mayflies in general are particularly sensitive to acidification (Bell & Nebeker, 1969; Fiance, 1978; Ormerod *et al.*, 1987), and Warnick & Bell (1969) showed that Fe^{2+} at a concentration of 0.32 mg L^{-1} had a toxic effect on mayflies. Other laboratory tolerance experiments have incorporated a wide range of pH and metal concentrations, which have not always reflected those, found in the habitats of the experimental animals. For instance, Witters *et al.*, (1984) exposed *Corixa punctata* from an acid boglake (pH 4.0, Al 0.2 mg L^{-1}) to up to 100 mg L^{-1} of Al and found that the elevated aluminium concentration resulted in reduced influx and a net loss of Na^+ and Cl^- ions. As well as being lethal, low pH may have sublethal effects on some benthic taxa, either physiologically or by reducing their food supply (Haines, 1981; Townsend *et al.*, 1983).

In addition to surface water, acid mine drainage can be expected to contaminate waters within the hyporheic zone. However, there has been little work done on the effects of acid mine drainage on hyporheic macroinvertebrate communities. Nelson *et al.* (1993) observed that in the upper Arkansas River in central Colorado, the hyporheic zone had as diverse a community as the surface substrate. However, the taxonomic composition of the two zones varied considerably. Forty-two taxa occurred in the hyporheos (95% insects) compared with 36 in the surface sediments (95% insects), but although Ostracoda were common in the hyporheos they were absent from the surface samples. In addition, capniid stoneflies *Capnia* sp., the mayfly *Paraleptophlebia* sp. and Chironomidae were much more common in the hyporheos than in surface sediment samples. At an acid mine drainage-affected site with elevated concentrations of iron and zinc, Nelson *et al.* (1993) reported that the hyporheos was dominated by Chironomidae and other Diptera whereas surface sediment samples were dominated by non-chironomid Diptera and Ephemeroptera.

The aim of the work reported in this chapter was to investigate the invertebrate communities of surface and hyporheic substrates in relation to physico-chemical conditions. The research focused on the following questions:

1) Does the diversity and abundance of surface and hyporheic invertebrate assemblages reflect differences in their physico-chemical environments ?
2) Is surface water invertebrate diversity and abundance similar to that found in the hyporheic zone ?
3) Do seasonal variations occur in invertebrate diversity and abundance ?
4) Do pH and iron tolerance levels of some New Zealand stream invertebrate species differ ?
5) Do populations of the same species from different stream environments differ in their pH and iron tolerance ?

METHODS

Except in March when there were 3
See Appendix 2.

Benthic faunal sampling

Quantitative sampling of benthic invertebrates was carried out at all ten sites with a Surber sampler (0.1 m^2; 0.5 mm pore size), seasonally between March 1998 and January 1999. Five samples were collected from each site. Streambed materials were thoroughly disturbed to a depth of about 5 cm and surfaces of the larger stones were wiped within the net to remove any attached fauna. All samples were bagged separately and preserved in 95% ethanol.

Benthic samples were washed over a 1 mm mesh sieve with fine particles being collected in a 125 μm mesh sieve beneath it. Animals were separated from sediment and detritus in the laboratory. Material collected on the larger sieve was placed in a white tray and picked through twice. Fine materials collected on the finer sieve were examined at up to 40 x magnification in a Bogorov tray, and all invertebrates were removed.

Animals were identified to the lowest taxonomic level possible and counted. Identifications were made using keys in Winterbourn and Gregson (1989).

Hyporheic faunal sampling

Hyporheic sampling was also carried out seasonally between March 1998 and January 1999 at all ten sites. Three replicate invertebrate samples were collected by pumping up to 5 litres of water from stainless-steel wells (diameter 16 mm) hammered (to a depth of 30 cm) into the stream bed (see Boulton et al., 1992). Water samples were passed through a 63 μm mesh net in the field and net contents were then rinsed into plastic containers and preserved with 95 % ethanol.

Animals were sorted from sediments in a Bogorov tray at up to 40 x magnification. All invertebrates were identified to the lowest possible taxonomic level, counted, and identified using keys in Chapman and Lewis (1976) and Winterbourn and Gregson (1989).

Laboratory tolerance experiments

Invertebrates used in tolerance experiments were collected from two sites on 7 November 1998. The collection sites are described in Chapter 2 (Figs 2.1 & 2.2) and were Site 2 (*Deleatidium* sp. and *Zelandobius confusus*) and Site 10 (*Deleatidium* sp., *Z.confusus* and *Pycnocentrella eruensis*). Invertebrates were collected with a Surber sampler and returned to the laboratory in large plastic bags containing water from the collection site. In order to minimize stress during transportation, all bags were kept as cool as possible inside a chilly bin containing ice. Samples were sorted in white plastic trays in the laboratory, and all animals were placed immediately into the appropriate experimental solution. Where possible, animals of medium-size were used throughout, so that comparisons of survival rate were not confounded by larval size. Water samples were analysed as described in Chapter 3.

All experimental solutions were set up at least 24 hours prior to collection of animals. They consisted of Christchurch tap water (pH 6.7, conductivity 129 $\mu S\ cm^{-1}$ and total iron < 0.01 mg L-1) (Table 4.2) whose pH (2.0 – 6.7) and iron concentration (1.45 to 11 mg L^{-1}) were modified (Table 4.1). pH was reduced by adding H_2SO_4 and iron was added as ferrous sulphate. Final concentrations of iron in test solutions were checked using Hach DR/2000 spectrophotometric procedures as described in Chapter 3. Twenty experimental treatments of pH and iron were used (Table 4.1). Three replicates of each treatment were run in each experiment, each containing ten animals. Polystyrene cups were used as experimental containers (see Stewart, 1993). Each 200 ml cup contained 100 ml of solution and all experimental treatments were kept in a 5 °C temperature controlled room with a 12h light:12h dark cycle. A 5 °C temperature controlled room was chosen because the cooler temperature slowed down mayfly (*Deleatidium*) activity and reduced stress on the animals in the otherwise still water. Animals were not fed during the 96 hour trials. Animals were checked every 12 hours, and dead individuals were removed. Checks were made at the beginning and end of the four day period to ensure that all chemical treatments were within at least 10% of their initial values.

Statistical Analysis

Data were analysed statistically for differences in abundance between sites and months using Kruskal-Wallis one-way non-parametric ANOVAs ($\alpha = 0.05$; Zar, 1984). Following a significant ANOVA, Tukey multiple comparison *a posteriori* tests were carried out to determine where significant differences lay. The Berger-Parker dominance index (Southwood, 1966) and Margalef's index (Winterbourn, 1980) were calculated to provide measures of evenness and richness of taxa.

The Berger-Parker index (d) was calculated as:

$$d = N_{max} / NT$$

where N = number of individuals of the single most abundant taxon and NT = the total number or individuals in all taxonomic groups. Margalef's index (d) was calculated as:

$$d = (S - 1) / \mathrm{Log}_e N$$

where S = the number of taxa and N = the number of individuals.

Relationships between invertebrate abundance and chemical variables were examined with Spearman rank correlations and linear regressions. Faunal assemblages at the 10 sites were compared using cluster analysis performed with the *PC-ORD* multivariate statistics package (McCune, 1995). Similarities among sites were determined using species presence/absence data and Sorensen's index (Southwood, 1966), and clustering was achieved with the average linkage method (McCune, 1995). Cluster analysis was carried out on data obtained in each season (March 1998, June 1998, September 1998 and January 1999) and for all four months combined.

Table 4.1 Combinations of pH and iron concentrations used in 96 hour, laboratory tolerance experiments with stream invertebrates.

Treatment	pH	Fe (mg L^{-1})
1 (Control)	6.7	< 0.01
2	5	< 0.01
3	3	< 0.01
4	2	< 0.01
5	6.7	1.45
6	5	1.45
7	3	1.45
8	2	1.45
9	6.7	3.9
10	5	3.9
11	3	3.9
12	2	3.9
13	6.7	6.6
14	5	6.6
15	3	6.6
16	2	6.6
17	6.7	11
18	5	11
19	3	11
20	2	11

Table 4.2 Chemistry of standard Christchurch tap water used in tolerance experiments. All values except pH and conductivity are mg L^{-1}.

pH	Conductivity ($\mu S\ cm^{-1}$ at 25°C)	Alkalinity	Al (total) dissolved	Fe (total)	Nitrate-Nitrogen	Reactive Phosphate
6.7	129	61	< 0.01	< 0.01	0.23	0.1

RESULTS

Surface fauna

Fifty-two taxa were collected from surface samples at the ten sites between March 1998 and January 1999 of which 45 were insects (Table 4.3). The Trichoptera was the most diverse order with 12 recognisable taxa, while 11 Plecoptera, 10 Diptera, 6 Ephemeroptera, 4 Coleoptera, 1 Mecoptera and 1 Neuroptera were also found. Non-insect groups collected were Oligochaeta, Amphipoda (*Paraleptamphopus subterraneus*), Copepoda (Harpacticoida), Ostracoda (*Herpetocypris pascheri*), Decapoda (*Paranephrops planifrons*), Platyhelminthes (*Neppia montana*), and Acarina (Table 4.3).

Control sites (5 and 10) had greater species richness (39 and 40, respectively) than sites affected strongly by acid mine drainage (14 – 20) due to slightly greater numbers of species in each taxonomic group (Table 4.4). Sites moderately affected by mine drainage (pH > 4.5) (Sites 2, 3 and 4) also had a relatively diverse range of taxa (27 – 39) (Table 4.4), consistent with the findings of Winterbourn & McDiffett (1996) and Winterbourn (1998) that a small number of aquatic insects characteristic of 'clean", stony streams occur down to a pH of about 4. At most sites, greatest species richness occurred within the Plecoptera and Diptera. Sites with pH < 4 (Sites 1, 6, 7, 8 and 9) had low numbers of Trichoptera and Ephemeroptera, and few or no coleopteran taxa (Table 4.4).

Taxa that occurred at the control sites (5 and 10) but not at sites affected by acid mine drainage included the crayfish *Paranephrops planifrons*, the mayfly *Zephlebia* sp. and the caddisflies *Aoteapsyche* sp. and *Pycnocentria sylvestris*. Taxa that occurred at all ten sites included Oligochaeta (Annelida), *Cristaperla fimbria* and *Spaniocerca zelandica* (Plecoptera), *Psilochorema* sp. (Trichoptera), Cer~~e~~atopogonidae, Chironomidae and Limoniinae (Diptera), and Acarina (Table 4.3). The Chironomidae was the only taxon that occurred in relatively high abundance at all ten sites, while *Deleatidium* was the most abundant taxon at both control sites (Table 4.5). *Cristaperla fimbria* and *Spaniocerca zelandica* were also relatively abundant at most sites. Overall, the control sites had the largest numbers of individuals and higher taxonomic richness than the acid sites (Table 4.5).

The Berger-Parker index indicates the dominance of a single major taxon at both Site 1 and Site 6 (*Spaniocercoides philpotti* and Chironomidae, respectively) (Table 4.5). Sites 4, 8, 9 and 10 were slightly less strongly dominated by a single taxon and Sites 2, 3, 5 and 7 even less so. In contrast, Margalef's index indicates high taxonomic richness at Sites 2, 5 and

Table 4.3 Occurrence of taxa in surface samples from each site (March 1998 to January 1999). + = present. Sites arranged from most to least acidic, left to right (and in subsequent tables).

	SITE									
	6	1	8	7	9	3	2	4	5	10
ANNELIDA										
Oligochaeta	+	+	+	+	+	+	+	+	+	+
PLATYHELMINTHES										
Neppia montana							+	+	+	+
CRUSTACEA										
Amphipoda										
Paraleptamphopus subterraneus	+		+		+	+	+	+	+	+
Copepoda										
Harpacticoida	+		+	+	+	+	+	+	+	+
Ostracoda										
Herpetocypris pascheri	+					+	+	+	+	+
Decapoda										
Paranephrops planifrons										+
INSECTA										
Ephemeroptera										
Austroclima sepia							+		+	+
Coloburiscus humeralis										+
Deleatidium spp.	+	+	+			+	+	+	+	+
Neozephlebia scita							+			+
Nesameletus sp.							+	+	+	+
Zephlebia sp.										+
Plecoptera										
Acroperla trivacuata								+	+	
Austroperla cyrene			+				+	+	+	+
Cristaperla fimbria	+	+	+	+	+	+	+	+	+	+
Megaleptoperla grandis						+	+		+	
Nesoperla fulvescens	+	+		+	+	+	+	+	+	+
Spaniocerca zelandica	+	+	+	+	+	+	+	+	+	+
Spaniocercoides philpotti	+	+		+		+	+	+	+	+
Stenoperla prasina		+				+	+	+	+	+
Taraperla sp.							+	+	+	
Zelandobius confusus Perla			+			+	+	+	+	+
Zelandobius fenestrata							+	+	+	
Trichoptera										
Aoteapsyche sp.									+	+
Hudsonema sp.				+						
Hydobiosella stenocerca		+					+	+	+	+
Hydrobiosis sp.	+	+		+	+	+	+	+	+	+
Oxyethira albiceps						+				
Philorheithrus agilis						+	+	+	+	+
Polyplectropus sp.						+	+	+	+	+
Psilochorema sp.	+	+	+	+	+	+	+	+	+	+
Pycnocentrella eruensis								+		+

Table 4.3 (continued)

	SITE									
	6	1	8	7	9	3	2	4	5	10
Pycnocentria sylvestris									+	
Triplectides obsoleta	+			+	+	+	+	+	+	+
Zelandopsyche maclellani							+	+		+
Coleoptera										
Hydraenidae		+				+	+	+	+	+
Hydrophilidae				+		+	+	+	+	+
Ptilodactylidae							+	+	+	+
Scirtidae				+			+	+	+	+
Diptera										
Austrosimulium sp.								+	+	+
Ceratopogonidae	+	+	+	+	+	+	+	+	+	+
Chironomidae	+	+	+	+	+	+	+	+	+	+
Eriopterini	+						+	+	+	+
Limoniinae	+	+	+	+	+	+	+	+	+	+
Muscidae	+	+			+			+		
Nothodixa sp.								+	+	+
Neocurupira tonnoiri										+
Paralimnophila skusei	+			+	+	+		+	+	+
Other Dipteran larvae	+	+	+	+	+	+	+	+	+	
Mecoptera										
Nannochorista philpotti							+			
Neuroptera										
Kempynus sp.				+		+	+	+		
Acarina	+	+	+	+	+	+	+	+	+	+

Table 4.4 Total number of invertebrate taxa collected in surface samples from each site (all dates combined).

	SITE									
TAXA	6	1	8	7	9	3	2	4	5	10
Ephemeroptera	1	1	1	-	-	1	4	2	3	6
Plecoptera	4	5	4	4	3	7	10	10	11	7
Trichoptera	3	3	1	4	3	6	7	8	7	9
Coleoptera	-	1	-	2	-	2	4	4	4	4
Diptera	7	5	4	5	6	5	5	9	8	8
Mecoptera	-	-	-	-	-	-	1	-	-	-
Neuroptera	-	-	-	1	-	1	1	1	-	-
Crustacea	3	-	2	1	2	3	3	3	3	4
Others *	2	2	2	2	2	2	3	3	3	3
Total	20	17	14	19	16	27	38	39	39	40

Others * = refers to the non insect groups Annelida, Platyhelminthes and Acarina

Table 4.5 Mean abundances (No. per 0.1 m^2) of the five most common invertebrate taxa found in the 10 streams, total abundance of invertebrates, total taxa (means of four seasons combined) collected at each site and Margalef's and Berger-Parker Indices for surface samples (March 1998 to January 1999).

	SITE									
	6	1	8	7	9	3	2	4	5	10
Chironomidae	67	16	10	11	11.7	21	24	78	42	26
Deleatidium sp.	0.7	2.4	0.2	-	-	4	11	19	50	100
Spaniocercoides philpotti	0.6	99	0.4	13	1.6	2.6	0.3	1.5	0.7	-
Cristaperla fimbria	0.7	0.4	0.3	1.6	0.3	2.5	8.8	5.6	19	3.3
Spaniocerca zelandica	1.4	0.3	0.7	1.9	1.7	2.3	4.8	2.4	14	5.4
Total Invertebrates	85	126	17.4	36	23	52	79	153	158	185
Total Taxa	7	6	5.3	7	5.4	10	13	16.5	16	18.5
Margalef's Index	2.6	2.7	2.2	2.9	2.7	3.8	4.8	4.7	4.8	4.8
Berger-Parker Index	0.79	0.79	0.57	0.36	0.51	0.4	0.3	0.51	0.32	0.54

10 but not at Sites 1, 6, 7, 8 or 9 (Table 4.5).

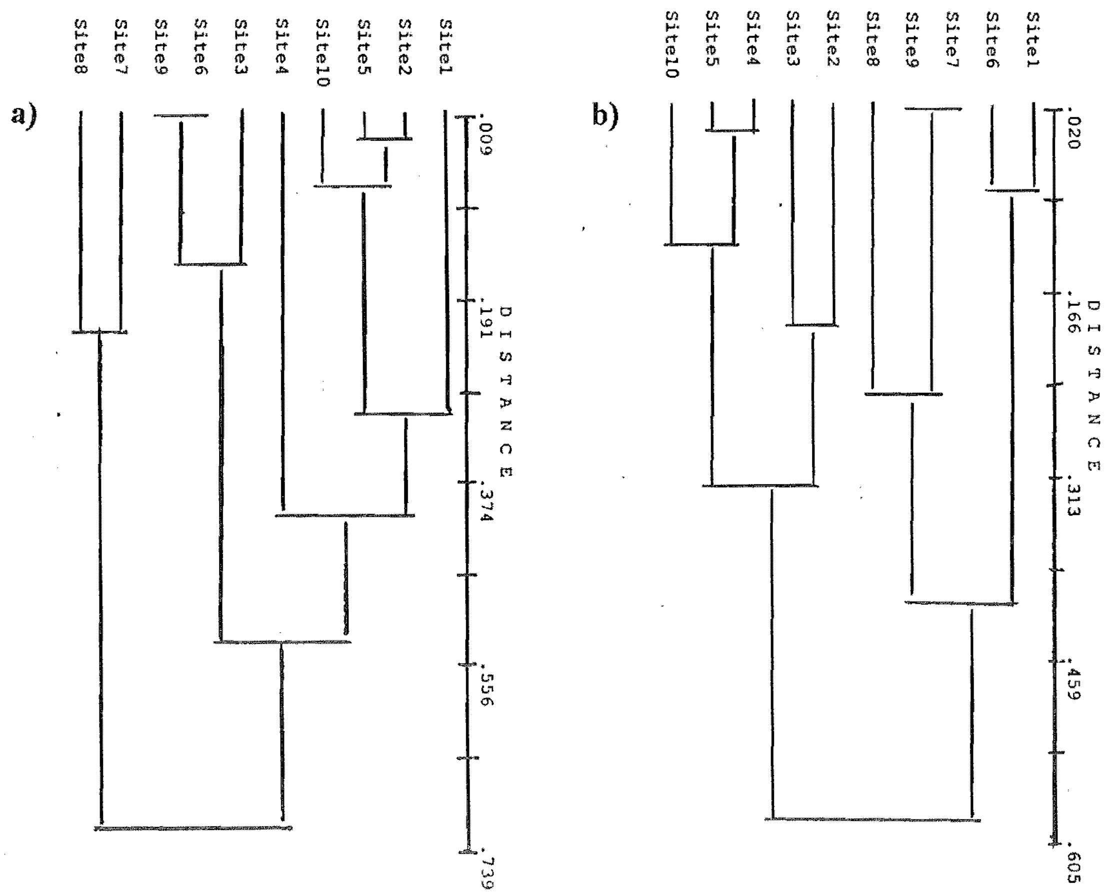
Clustering with Sorensen's index confirmed the high degree of faunal similarity between most acidic sites (Sites 1, 3, 6, 9, 7 and 8) in all four months combined (Fig 4.1e). Sites 2 and 4 (pH > 4.5) and Sites 5 and 10 (controls) had similar faunal composition which differed considerably from the most acidic sites (Fig 4.1e). When all four months were considered separately, results similar to those with the combined data were found although clustering of some sites varied seasonally. Generally, highly acidic sites (pH < 4.5) were clustered apart from the control sites and acidic sites with pH > 4.5, which had similar faunal composition (Fig 4.1a, b, c & d).

The number of individuals present at a site was significantly and positively correlated with stream water pH, while a significant, negative relationship was found between the number of individuals and stream water total iron concentration (Fig 4.2a & e). The three other chemical parameters measured (conductivity, alkalinity and total dissolved aluminium) were not significantly correlated with the number of individuals found, although weak patterns were apparent. Conductivity and total dissolved aluminium levels showed negative trends with the number of individuals, while alkalinity displayed a positive trend (Figs 4.2b, c & d).

Mean numbers of individuals differed significantly among sites in March, June, September and January and when numbers of invertebrates in the four months were combined (Fig 4.3) ($P < 0.001$). In all months, high abundances were found at the control sites whereas Sites 7, 8 and 9 had the fewest individuals (Fig 4.3).

Mean numbers of invertebrates at all ten sites combined did not differ significantly with season ($P = 0.306$) (Fig 4.4a), and neither did abundances of Chironomidae and *Deleatidium* (Figs 4.4c & d). The abundance of *Spaniocercoides philpotti* did differ significantly with season ($P < 0.001$) however, as it was absent in March but abundant in September and June (Fig 4.4e). Like total individuals, the numbers of taxa taken at all sites did not differ significantly over time (Fig 4.4b), with mean numbers found in the 4 months ranging from 10.1 to 11.8.

In summary, many of the taxa that were found in this study appeared to have broad habitat requirements, at least in terms of the physico-chemical factors measured in this study. Many animals appeared to be able to tolerate low pH (Table 4.6) and most of the Plecoptera and Diptera that were found at a minimum of four sites were able to tolerate pH down to at



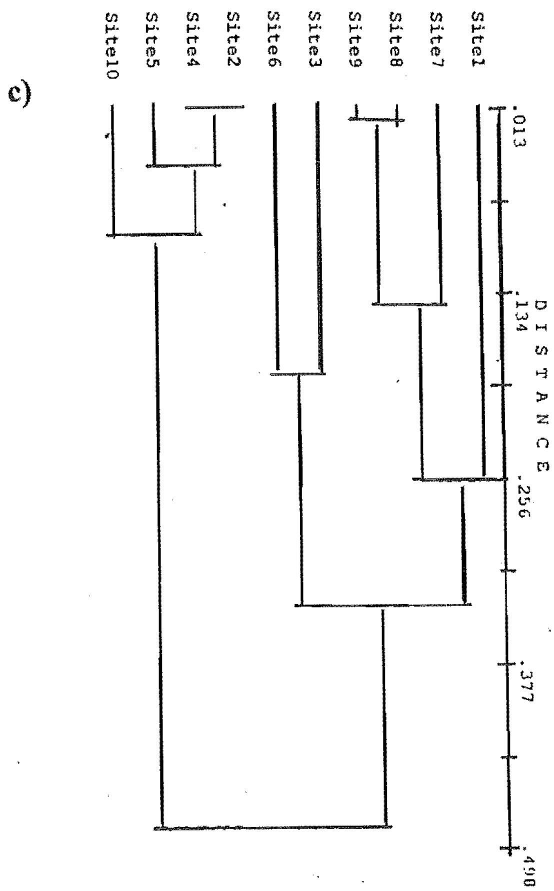
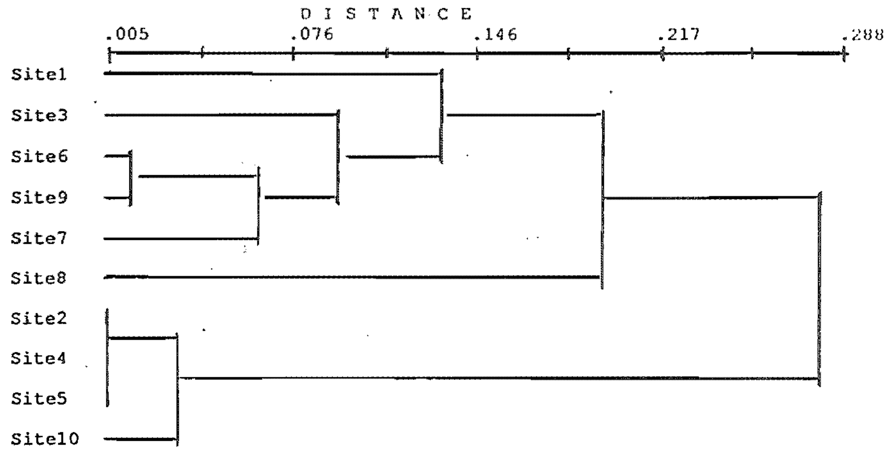


Fig 4.1 Average linkage clustering of the 10 sites using Sorensen's Index, **a)** March, **b)** June, **c)** September, **d)** January and **e)** all 4 months. N.B. Missing value for Site 1 in January due to nil flow.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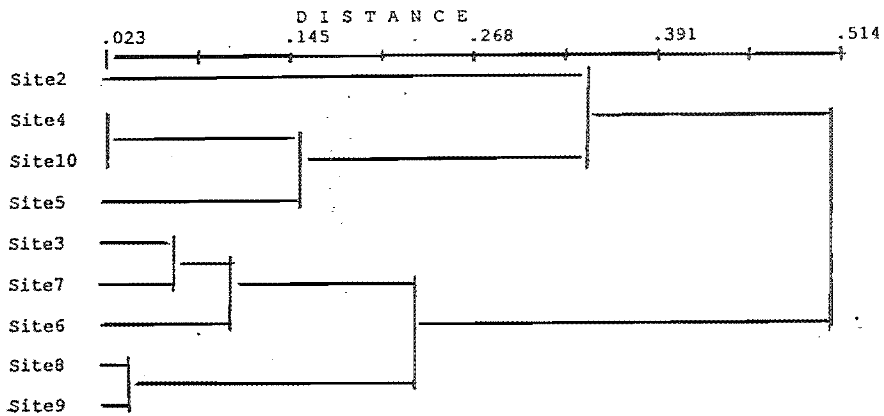
.005
.076
.146
.217
.288
Site1
Site3
Site6
Site9
Site7
Site8
Site2
Site4
Site5
Site10

e)


DISTANCE
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.145
.268
.391
.514
Site2
Site4
Site10
Site5
Site3
Site7
Site6
Site8
Site9

d)

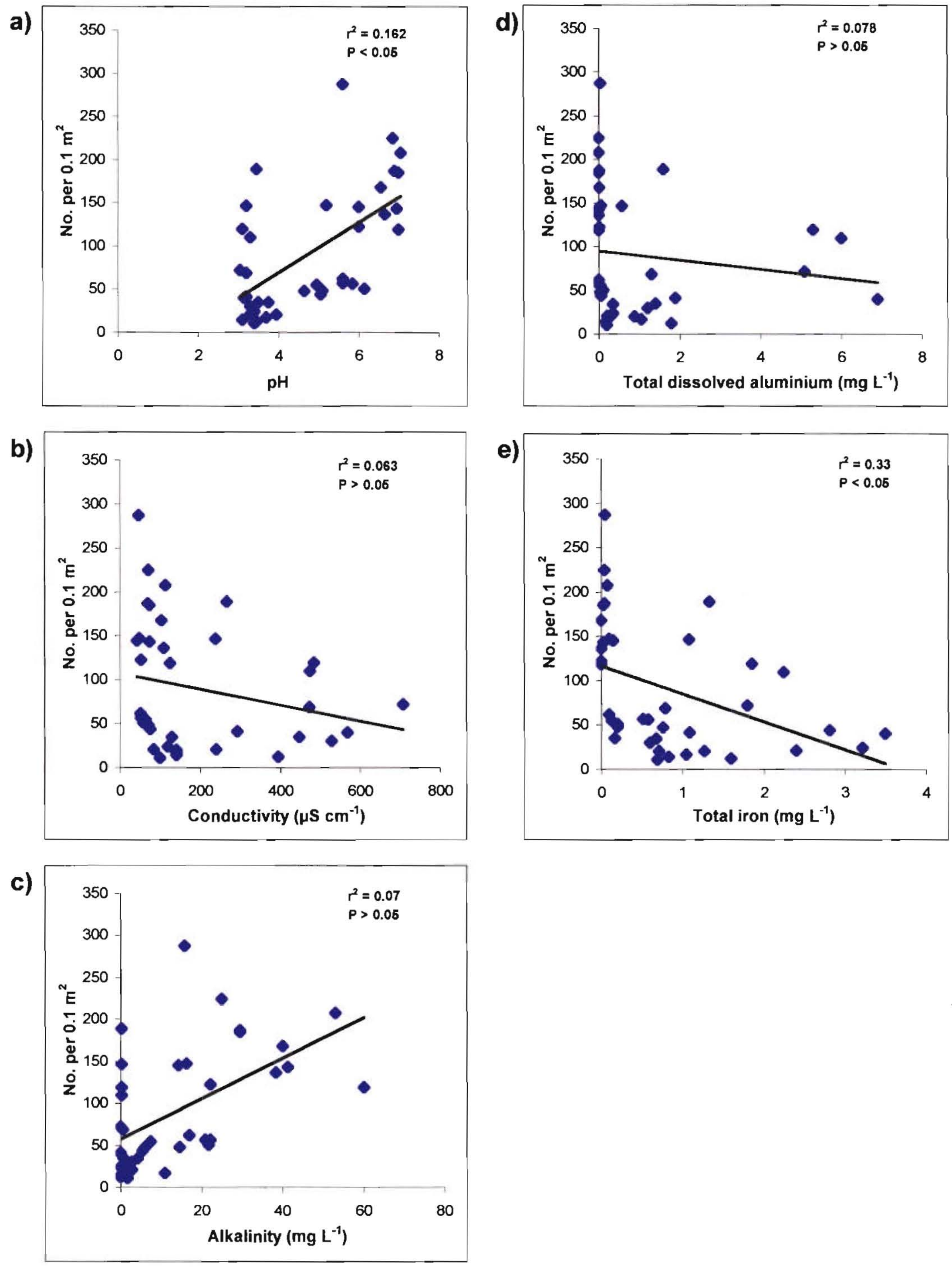


Fig 4.2 Relationships between the numbers of individuals (per 0.1 m^2) collected in surface samples and **a)** pH, **b)** conductivity, **c)** alkalinity, **d)** total dissolved aluminium and **e)** total iron (seasonal means).

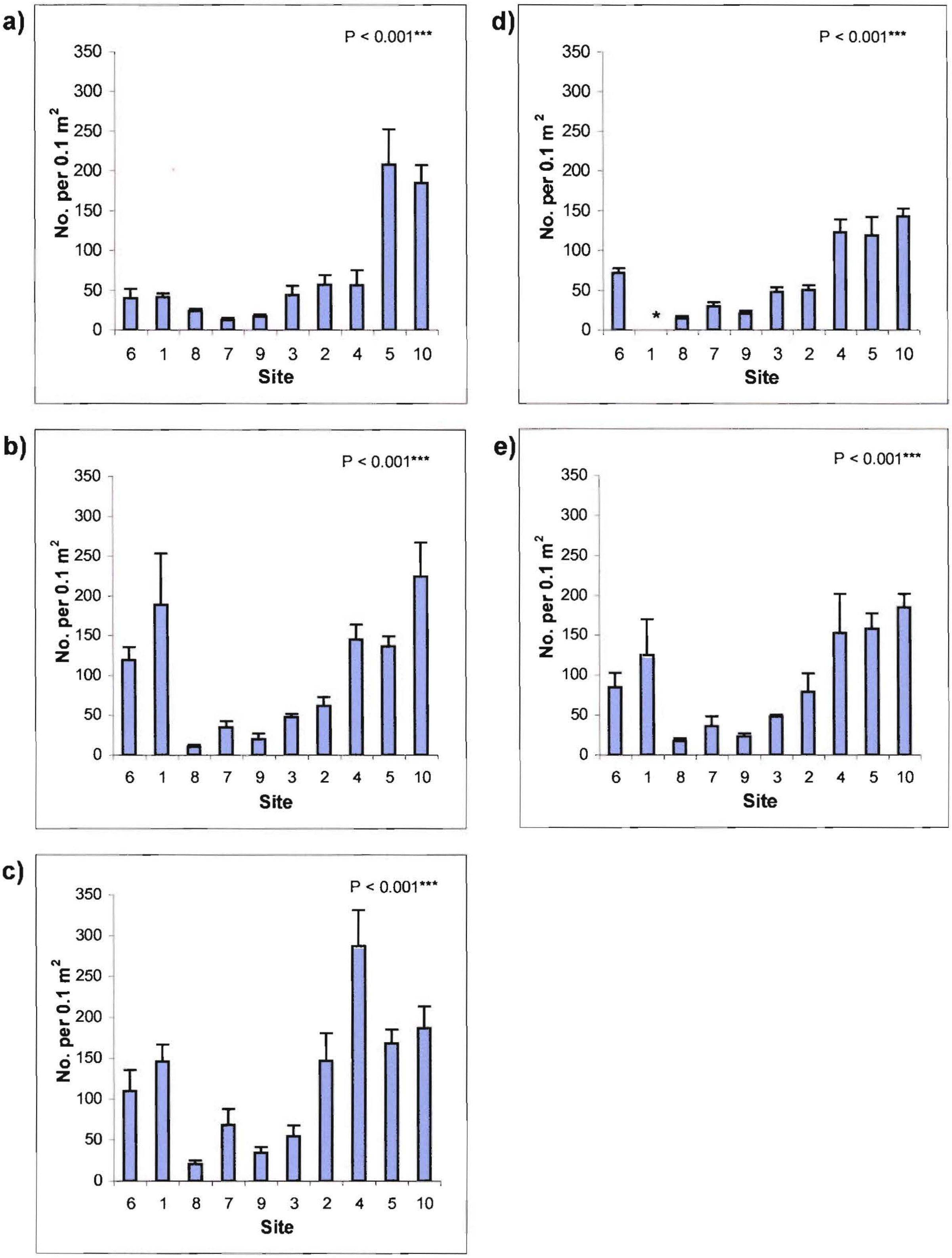


Fig 4.3 Abundances of invertebrates (mean ± 1 SE) at each site in **a)** March, **b)** June, **c)** September, **d)** January and **e)** all four months. * denotes no sample taken (nil flow). P values are for Kruskal-Wallis analysis of variance among results. (Sites arranged from most to least acidic, left to right)

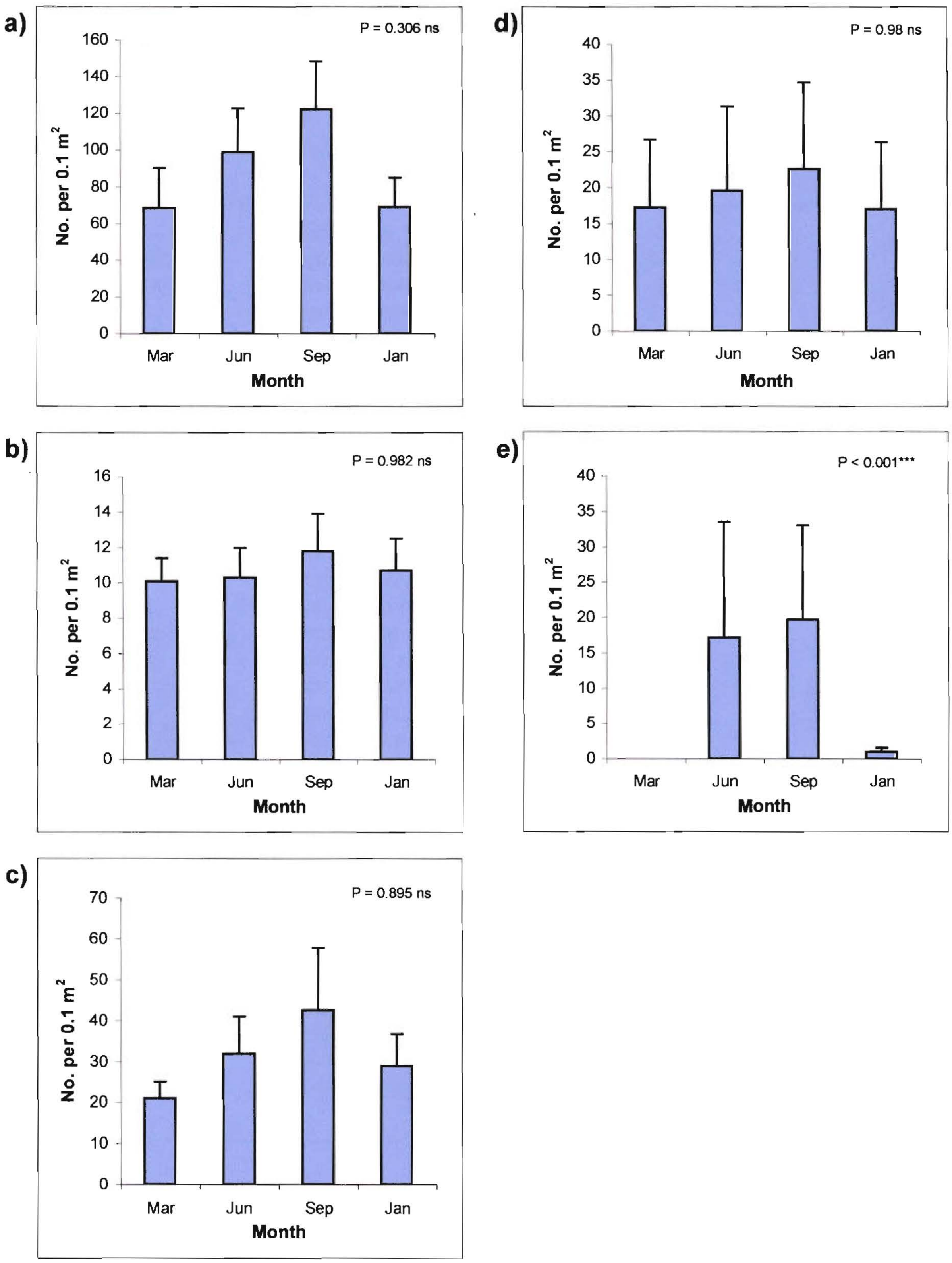


Fig 4.4 Abundances of **a)** total invertebrates, **b)** total taxa, **c)** Chironomidae, **d)** *Deleatidium* spp. and **e)** *Spaniocercoides philpotti* collected in 4 months (mean ± 1 SE). Note that the scale of the y-axes varies. P values are for Kruskal-Wallis analyses of variance.

Table 4.6 Distribution of 34 taxa in relation to stream water pH. Only taxa found at a minimum of 4 sites are shown.

		pH	
		Min	Max
Annelida	Oligochaeta	3	7.1
Platyhelminthes	*Neppia montana*	5.7	7.1
Amphipoda	*Paraleptamphopus subterraneus*	3	7.1
Copepoda	Harpacticoida	2.9	7.1
Ostracoda	*Herpetocypris pascheri*	3.4	7.1
Ephemeroptera	*Deleatidium* spp.	3	7.1
	Nesameletus sp.	5.1	7.1
Plecoptera	*Austroperla cyrene*	3	7.1
	Cristaperla fimbria	2.9	7.1
	Megaleptoperla grandis	4.8	6.8
	Nesoperla fulvescens	3.5	6.9
	Spaniocerca zelandica	3	7.1
	Spaniocercoides philpotti	2.9	6.9
	Stenoperla prasina	3.4	7.1
	Zelandobius confusus	3.5	7.1
Trichoptera	*Hydobiosella stenocerca*	3.4	7.1
	Hydrobiosis sp.	2.9	7.1
	Philorheithrus agilis	4.8	7.1
	Polyplectropus sp.	4.8	7
	Psilochorema sp.	2.9	7.1
	Triplectides obsoleta	3.2	7
Coleoptera	Hydraenidae	3.3	7.1
	Hydrophilidae	3.3	7.1
	Ptilodactylidae	5.1	7.1
	Scirtidae	3.3	7.1
Diptera	Ceratopogonidae	3	7
	Chironomidae	2.9	7.1
	Eriopterini	3.2	7.1
	Limoniinae	3	7
	Muscidae	3.2	5.9
	Paralimnophila skusei	2.9	7
	Other Dipteran larvae	2.9	6.6
Neuroptera	*Kempynus* sp.	3.5	6
Acarina		2.9	7.1

least 3.5. Nine animals were found in waters with pH as low as 2.9 (Table 4.6).

Hyporheic fauna

Sixteen taxa were collected in hyporheic samples from the ten sites between March 1998 and January 1999 of which ten were insects (Table 4.7). The Plecoptera was the most diverse order with 4 species, while 3 Diptera, 2 Ephemeroptera and 1 trichopteran species were also found. Non-insect groups collected were Oligochaeta, Amphipoda, Copepoda, Ostracoda and Acarina (Table 4.7).

Control sites (5 and 10) and sites with pH > 4.5 (Sites 2, 3, and 4) had greater species richness than sites more strongly affected by acid mine drainage, largely due to a greater number of plecopteran and crustacean taxa (Table 4.8). At the most acidic sites the greatest diversity occurred within the Diptera and Crustacea. Ephemeroptera were present at very low densities in the hyporheic zone, and were found only at Sites 2, 3 and 10 (Table 4.8). Hyporheic samples were dominated by Crustacea whose species richness was slightly greater than in surface samples. Coleoptera, Mecoptera and Neuroptera were all found in surface samples but were not present in hyporheic samples. No taxa occurred solely in the hyporheic zone at control sites, however, *Ameletopsis* sp., *Deleatidium* sp. and *Stenoperla prasina* occurred only at sites with pH > 4.5 (Table 4.7).

Taxa that occurred at all ten sites included Oligochaeta, Harpacticoida, Chironomidae, and Acarina (Table 4.7). Harpacticoid copepods were the only invertebrates that were moderately abundant in hyporheic samples from all ten sites, and they were the most abundant animals at both control sites (Table 4.9). Oligochaeta, Chironomidae and Acarina also occurred at all sites in Surber samples, while Chironomidae and *Cristaperla fimbria* were two of the five most abundant invertebrate taxa in both surface and hyporheic samples. Overall, control sites and sites with pH > 4.5 had a greater abundance of hyporheic individuals and greater taxonomic richness than the more acid sites (Table 4.9).

Berger-Parker indices emphasised the dominance of a single major taxon (Harpacticoida) at both Sites 1 and 3 (Table 4.9). However, indices were lower at sites 2, 5 and 8, and lower still at Sites 4, 6, 7, 9 and 10 (Table 4.9). In contrast, Margalef's index reflected the higher diversity (richness) at Sites 2, 4, 5 and 10 than at Sites 1, 3, 6, 7, 8 and 9 (Table 4.9).

Clustering with Sorensen's index of similarity indicated a high degree of taxonomic

Table 4.7 Occurrence of taxa collected in hyporheic samples at each site from March 1998 to January 1999. + = present. Sites arranged from most to least acidic, left to right (and in subsequent tables).

	6	1	7	8	9	4	2	3	5	10
ANNELIDA										
Oligochaeta	+	+	+	+	+	+	+	+	+	+
CRUSTACEA										
Amphipoda										
Paraleptamphopus subterraneus		+	+	+		+	+	+	+	+
Copepoda										
Harpacticoida	+	+	+	+	+	+	+	+	+	+
Cyclopoida		+		+		+	+		+	+
Ostracoda										
Herpectocypris pascheri		+				+	+	+	+	+
INSECTA										
Ephemeroptera										
Ameletopsis sp.							+	+		+
Deleatidium sp.							+			
Plecoptera										
Cristaperla fimbria	+	+				+	+	+	+	+
Spaniocerca zelandica			+	+	+	+	+	+	+	+
Stenoperla prasina						+	+			+
Spaniocercoides philpotti		+					+			
Trichoptera										
Hudsonema sp.	+		+	+			+	+		+
Diptera										
Chrionomidae	+	+	+	+	+	+	+	+	+	+
Limonidae			+				+			
Dipteran larvae	+		+	+		+	+	+	+	+
Acarina	+	+	+	+	+	+	+	+	+	+

Table 4.8 Total numbers of taxa collected in hyporheic samples at each site.

	SITE									
	6	1	7	8	9	4	2	3	5	10
Ephemeroptera	-	-	-	-	-	-	2	1	-	1
Plecoptera	1	2	1	1	1	3	4	2	2	3
Trichoptera	1	-	1	1	-	-	1	1	-	1
Diptera	2	-	3	2	1	2	3	2	2	2
Crustacea	1	4	2	3	1	4	4	3	4	4
Others *	2	2	2	2	2	2	2	2	2	2
Total	7	8	9	9	5	11	16	11	10	13

Others * = refers to the non-insect groups Annelida and Acarina

Table 4.9 Mean abundance (No. per 5L) of the five most common invertebrate taxa found at the 10 sites, total abundances of invertebrates, total taxa (means of four seasons combined) and Margalef's and Berger-Parker Indices for hyporheic samples (March 1998 to January 1999).

	SITE									
	6	1	7	8	9	4	2	3	5	10
Harpacticoida	1.8	11	4.3	4.5	2	1.9	12.7	26	7	32
Chironomidae	2	1	1.2	0.8	1.5	2.3	5	3.4	2.2	8.6
Oligochaeta	0.8	0.8	2.2	1	2	1	2	3.7	2.1	8
Cristaperla fimbria	0.3	1.4	-	-	-	0.8	1.9	1.4	0.8	6
Paraleptamphopus subterraneus	-	0.5	0.7	0.6	0.2	0.8	1.3	2.1	1.2	4.2
Total Invertebrates	5.9	17.2	10.2	7.9	6.4	10.1	26	39	17.1	76
Total Taxa	3	4.2	4.4	3.1	3.2	5.6	7.6	6.7	6.3	9.2
Margalef's Index	1.13	1.12	1.46	1.02	1.19	1.99	2.03	1.56	1.91	2.01
Berger-Parker Index	0.34	0.64	0.4	0.57	0.32	0.22	0.43	0.65	0.47	0.33

similarity at the control sites (5 and 10) and sites with pH > 4.5 (Sites 2, 3 and 4) in all four months combined (Fig 4.5e). However, seasonal variations in taxonomic similarity meant that clusters differed each month (Fig 4.5a, b, c & d).

Numbers of individuals were significantly and positively correlated with pH and alkalinity of the hyporheic water (Fig 4.6a & c) and significantly and negatively correlated with conductivity, total dissolved aluminium and total iron (Fig 4.6b, d & e). In contrast, numbers of individuals in Surber samples were significantly correlated only with pH and total iron.

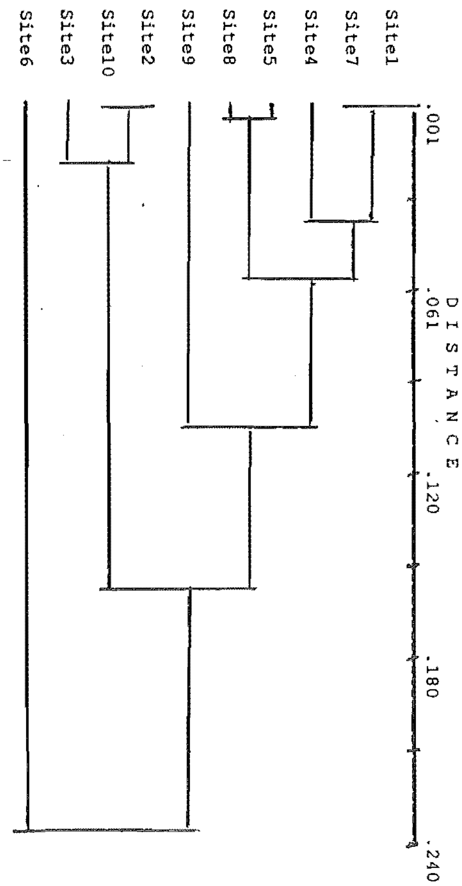
Mean numbers of individuals differed significantly among sites in March, June, September and January and also when data for all four months were combined ($P < 0.001$) (Fig 4.7). In all months, the highest abundances were found at control site 10, while Sites 4, 6, 7, 8 and 9 had fewest individuals (Fig 4.7). Mean numbers of invertebrates at all ten sites combined did not differ significantly with season ($P = 0.994$) (Fig 4.8a), and neither did abundances of Harpacticoida, Chironomidae and Oligochaeta (Fig 4.8).

Overall, my results indicate that the composition of the invertebrate faunas of the hyporheic and surface zones of the streambeds differed in a number of ways, most obvious being the lower representation of insect taxa in the hyporheic (48 %) than in surface (82 %) samples. As in the latter, many taxa inhabiting the hyporheic zone had broad habitat requirements and occurred over a wide pH range, extending down to pH 2.8 in some cases (Table 4.10).

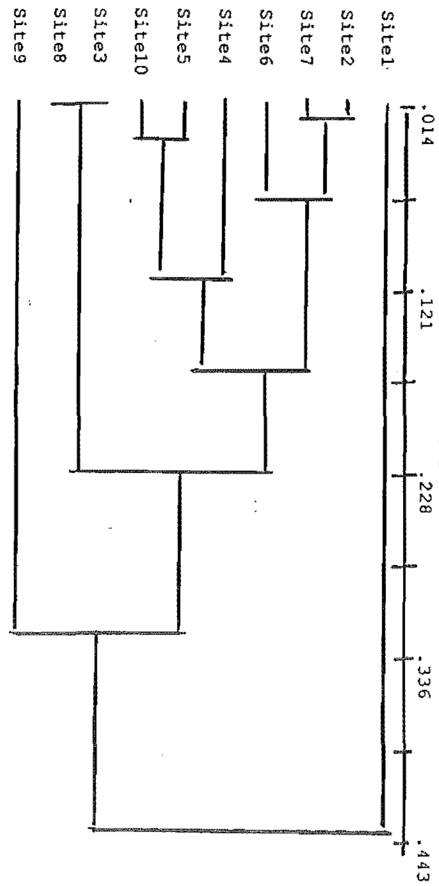
Table 4.10 Distributions of 11 taxa in relation to hyporheic water pH. Only taxa found at a minimum of 4 sites are shown.

		pH	
		Min	Max
Annelida	Oligochaeta	2.8	6.9
Amphipoda	*Paraleptamphopus subterreneus*	3.1	6.9
Copepoda	Harpacticoida	2.8	6.9
	Cyclopoida	3.1	6.9
Ostracoda	*Herpetocypris pascheri*	3.4	6.9
Plecoptera	*Cristaperla fimbria*	3.1	6.9
	Spaniocerca zelandica	3.1	6.9
Trichoptera	*Hudsonema* sp.	3.1	6.9
Diptera	Chironomidae	2.8	6.9
	Other Dipteran larvae	2.8	6.9
Acarina		2.8	6.9

a)



b)



c)

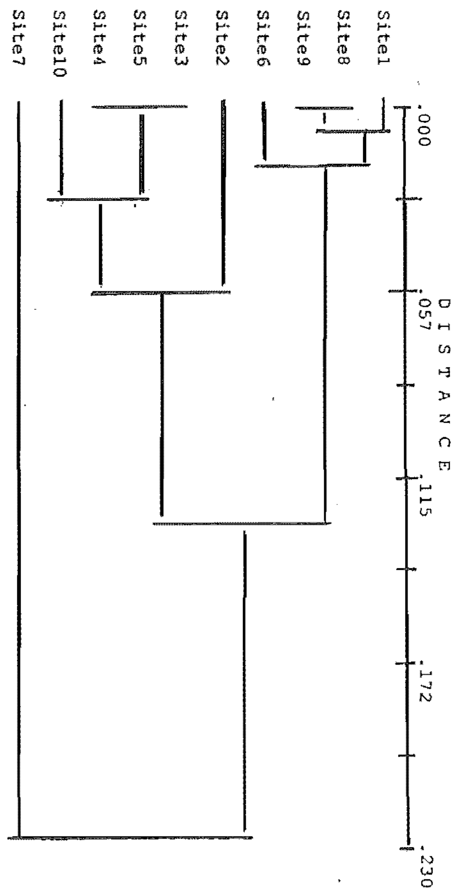
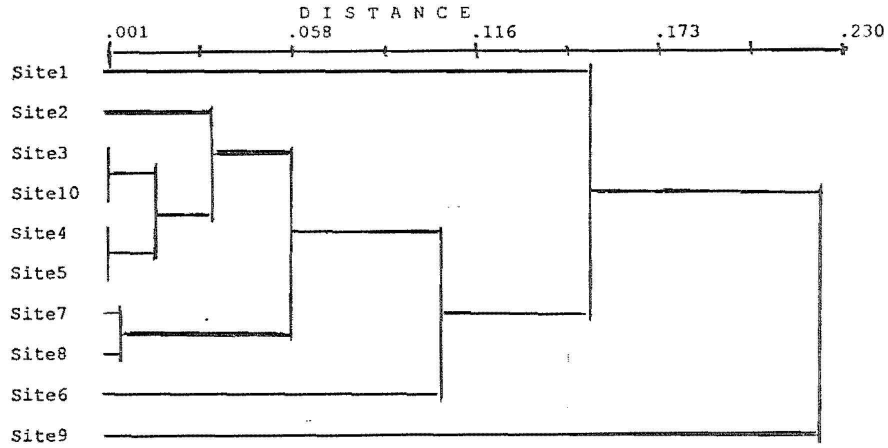


Fig 4.5 Average linkage clustering of the 10 sites using Sorensen's Index, **a)** March, **b)** June, **c)** September, **d)** January and **e)** all 4 months combined.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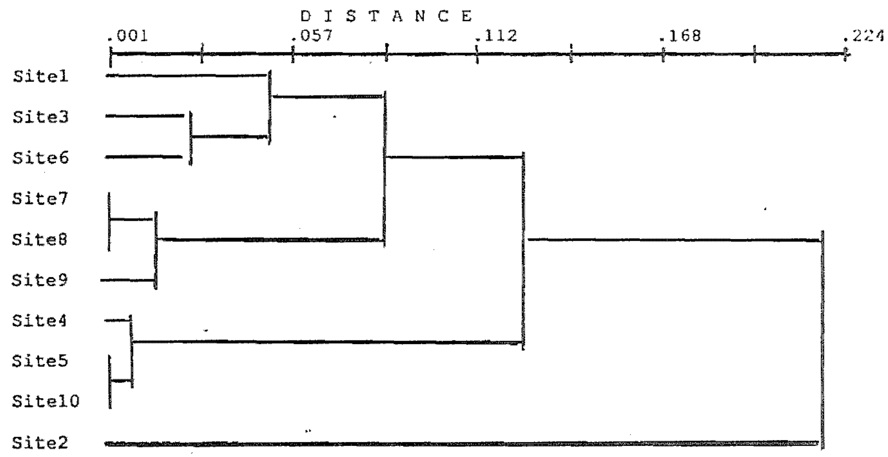
Site1
Site2
Site3
Site10
Site4
Site5
Site7
Site8
Site6
Site9

e)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Site3
Site6
Site7
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Site9
Site4
Site5
Site10
Site2

d)

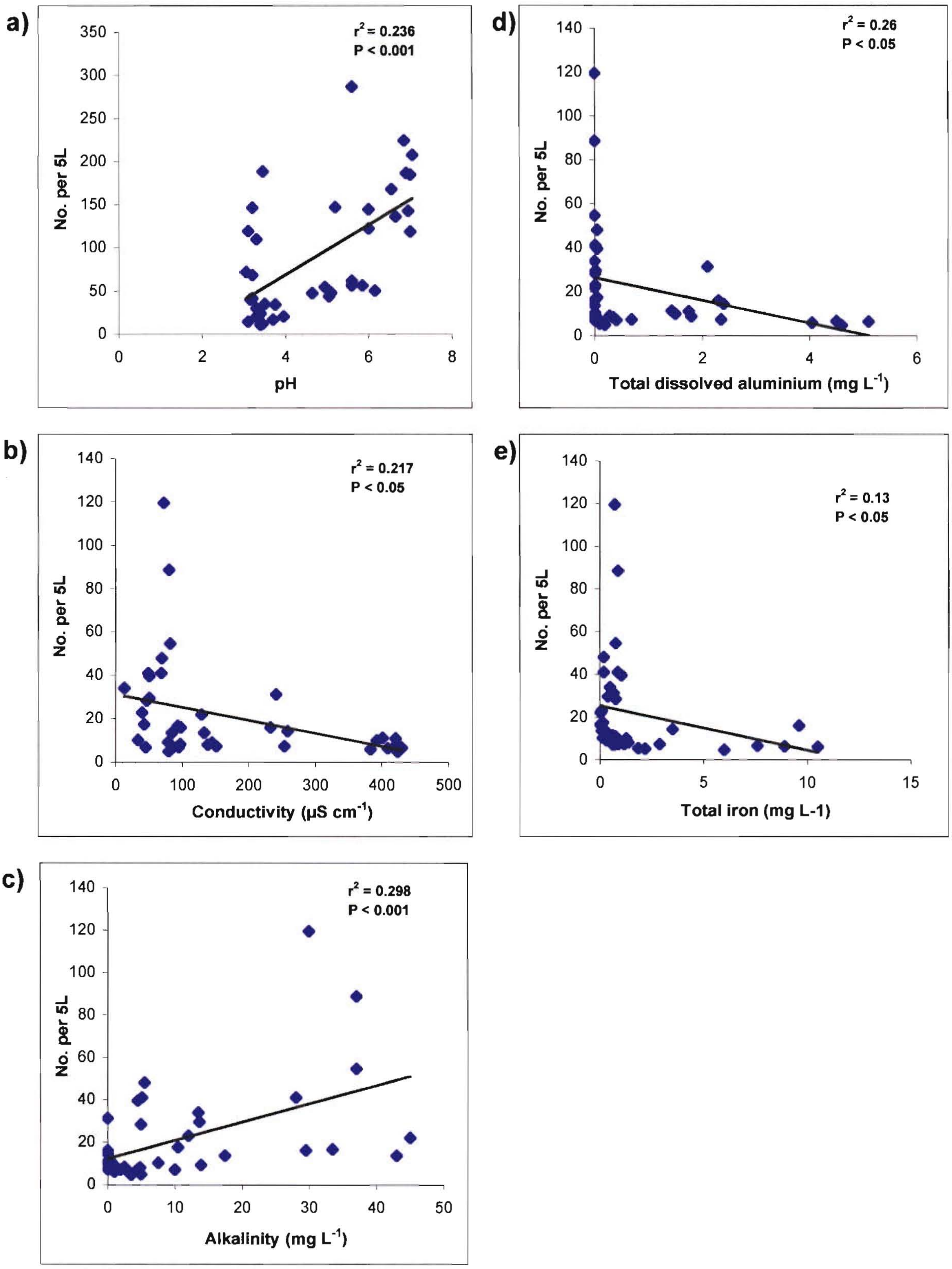


Fig 4.6 Relationships between numbers of individuals (per 5L water) collected in hyporheic samples and **a)** pH, **b)** conductivity, **c)** alkalinity, **d)** total dissolved aluminium and **e)** total iron (seasonal means).

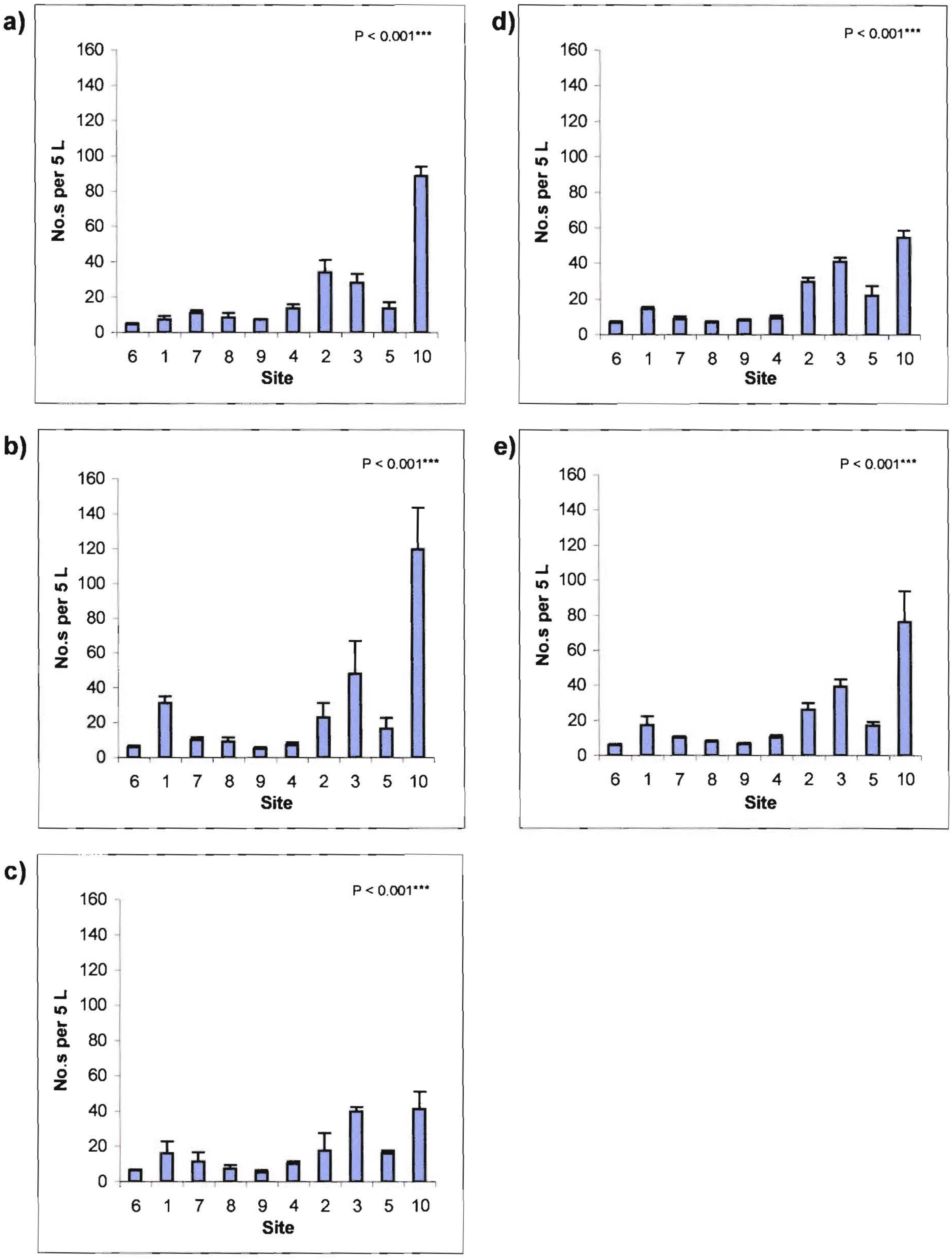


Fig 4.7 Abundances of invertebrates (mean ± 1 SE) at each site in **a)** March, **b)** June, **c)** September, **d)** January and **e)** all four months. P values are for Kruskal-Wallis analyses of variance. (Sites arranged from most to least acidic, left to right)

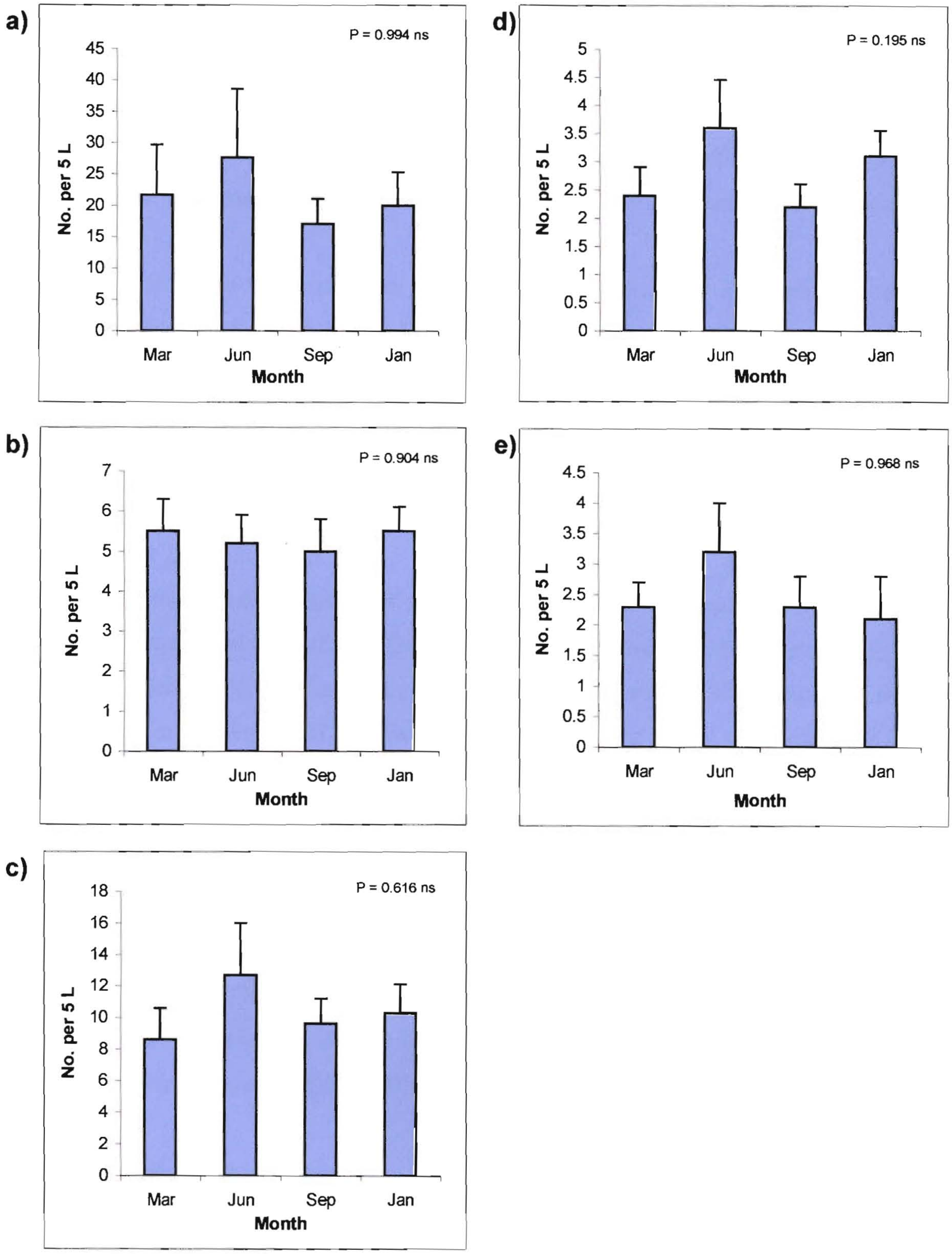


Fig 4.8 Abundances of **a)** total invertebrates, **b)** total taxa, **c)** Harpacticoida, **d)** Chironomidae and **e)** Oligochaeta collected in hyporheic samples in 4 months (mean ± 1 SE).
P values are for Kruskal-Wallis analyses of variance.
Note that the scales of y-axes differ.

pH and iron tolerance

Acute toxicity testing was carried out on three species: an ephemeropteran (*Deleatidium* sp.), a plecopteran (*Zelandobius confusus*) and a trichopteran (*Pycnocentrella eruensis*) collected from a circumneutral pH stream, and *Deleatidium* and *Zelandobius confusus* from a stream contaminated by moderately low concentrations of acid mine drainage.

Water from the collection sites differed in pH, conductivity and iron concentration. Thus, pH ranged from 5.1 (Site 2) to 7.1 (Site 10), conductivity from 41 $\mu S\ cm^{-1}$ (Site 2) to 79 $\mu S\ cm^{-1}$ (Site 10), and total iron from < 0.04 $mg\ L^{-1}$ (Site 10) to 0.52 $mg\ L^{-1}$ (Site 2) (Table 3.2).

<u>pH Tolerance</u>

Ephemeroptera: *Deleatidium* sp.

Deleatidium from circumneutral and acidic sites had < 10 % mortality at pH 5 and 6.7, whereas all animals died at pH 2. All *Deleatidium* from the circumneutral site also died at pH 3 (Fig 4.9a), whereas 55 % of mayflies from the acid site survived 96 hr exposure at pH 3. This difference in survival at pH 3 between the two groups was highly significant ($P < 0.001$).

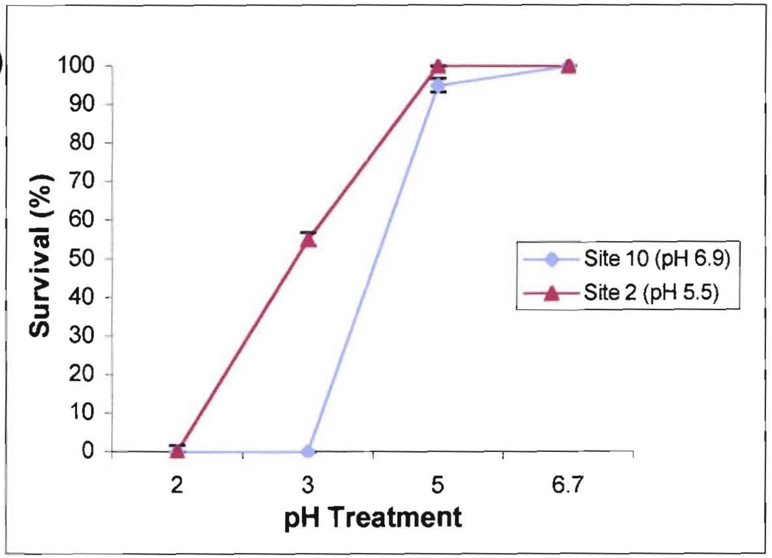
Plecoptera: *Zelandobius confusus*

Zelandobius confusus from circumneutral and acidic sites had < 10 % mortality at pH 5 and 6.7, whereas all animals died at pH 2. At pH 3, 75 % of *Zelandobius confusus* from the circumneutral site died, whereas 85 % of stoneflies from the more acidic site survived 96 hr exposure (Fig 4.9 b). The difference in survival of the two groups was highly significant at pH 3 ($P < 0.001$). Tolerance of low pH was significantly different between *Zelandobius confusus* and *Deleatidium* ($P < 0.05$) with a higher percentage of mayflies suffering mortality.

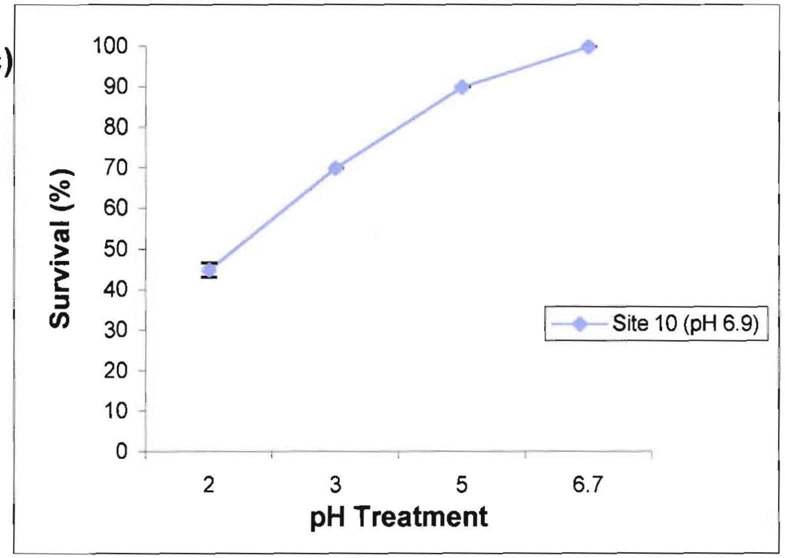
Trichoptera: *Pycnocentrella eruensis*

Pycnocentrella eruensis was the only caddisfly species found in sufficient abundance at the circumneutral site to enable tolerance experiments to be carried out. However, it was not present in high enough numbers at acidic sites for enough larvae to be collected. *P.eruensis* was significantly more tolerant of low pH than *Zelandobius confusus* and

a)



c)



b)

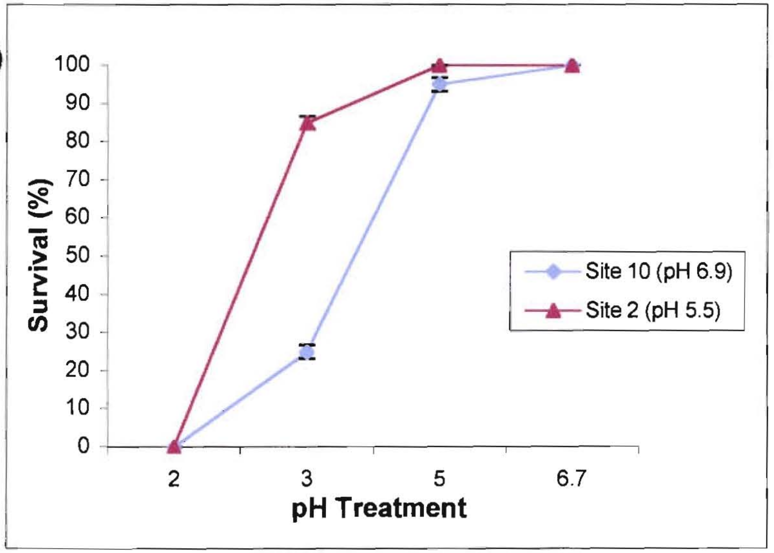


Fig 4.9 Percentage survival (± 1 SE) of animals exposed to different pH levels.
a) *Deleatidium* collected from an acid site (Site 2; pH 5.5) and a circumneutral site (Site 10; pH 6.9).
b) *Z.confusus* collected from an acid site (Site 2; pH 5.5) and a circumneutral site (Site 10; pH 6.9).
c) *P.eruensis* collected from a circumneutral site (Site 10; pH 6.9).

Deleatidium with 70 % of larvae surviving at pH 3 and 45 % at pH 2 (Fig 4.9c).

pH*Fe Tolerance

Ephemeroptera: *Deleatidium*

Over 70 % of *Deleatidium* larvae from circumneutral and acidic streams survived in all pH*Fe treatments at both pH 5 and pH 6.7 (Fig 4.10c, d). Mayflies from circumneutral streams survived significantly better at pH 3 when iron was added at concentrations of 1.45 to 6.6 mg L^{-1}, but did not survive at concentrations of 11 mg L^{-1} ($P < 0.001$) (Fig 4.10b). Fewer than 8 % of larvae survived in any treatment at pH 2. Differences in survival of larvae from circumneutral and acidic sites were highly significant ($P < 0.001$), with larvae from acid sites having greater survival.

Plecoptera: *Zelandobius confusus*

Zelandobius confusus from both circumneutral and acidic sites survived in all pH*Fe treatments at both pH 5 and pH 6.7 (Fig 4.11c, d). Like *Deleatidium*, *Zelandobius confusus* survived significantly better at pH 3 when iron was added in concentrations up to 6.6 mg L^{-1} but not 11 mg L^{-1} ($P < 0.05$) (Fig 4.11b). Fewer than 10 % of larvae survived in any treatment at pH 2. The difference in survival between animals from the two sites was highly significant ($P < 0.001$), with more of those from acid sites surviving.

DISCUSSION

In the Northern Hemisphere, reductions in species richness have been reported at about pH 5.7 in streams affected by acid precipitation (Sutcliffe & Hildrew, 1989). Below this threshold, mayflies, some crustaceans and molluscs tend to disappear or become scarce. However, contrasting results were found by Winterbourn & Collier (1987) who recorded little change in species composition or richness that could be associated with changes in water chemistry in New Zealand brown water streams above a pH of about 4.5.

In my study, ten streams that incorporated a wide range of pH, Al and Fe concentrations were surveyed. Based on species richness, moderately acidic (pH 4.5 – 6.0) and circumneutral (pH > 6.5) streams provided the most favourable habitats for aquatic invertebrates, and the very acidic (pH < 4.5) streams provided the least favourable habitats. One stream (Site 3) with moderate acidity also provided an unfavourable habitat, primarily

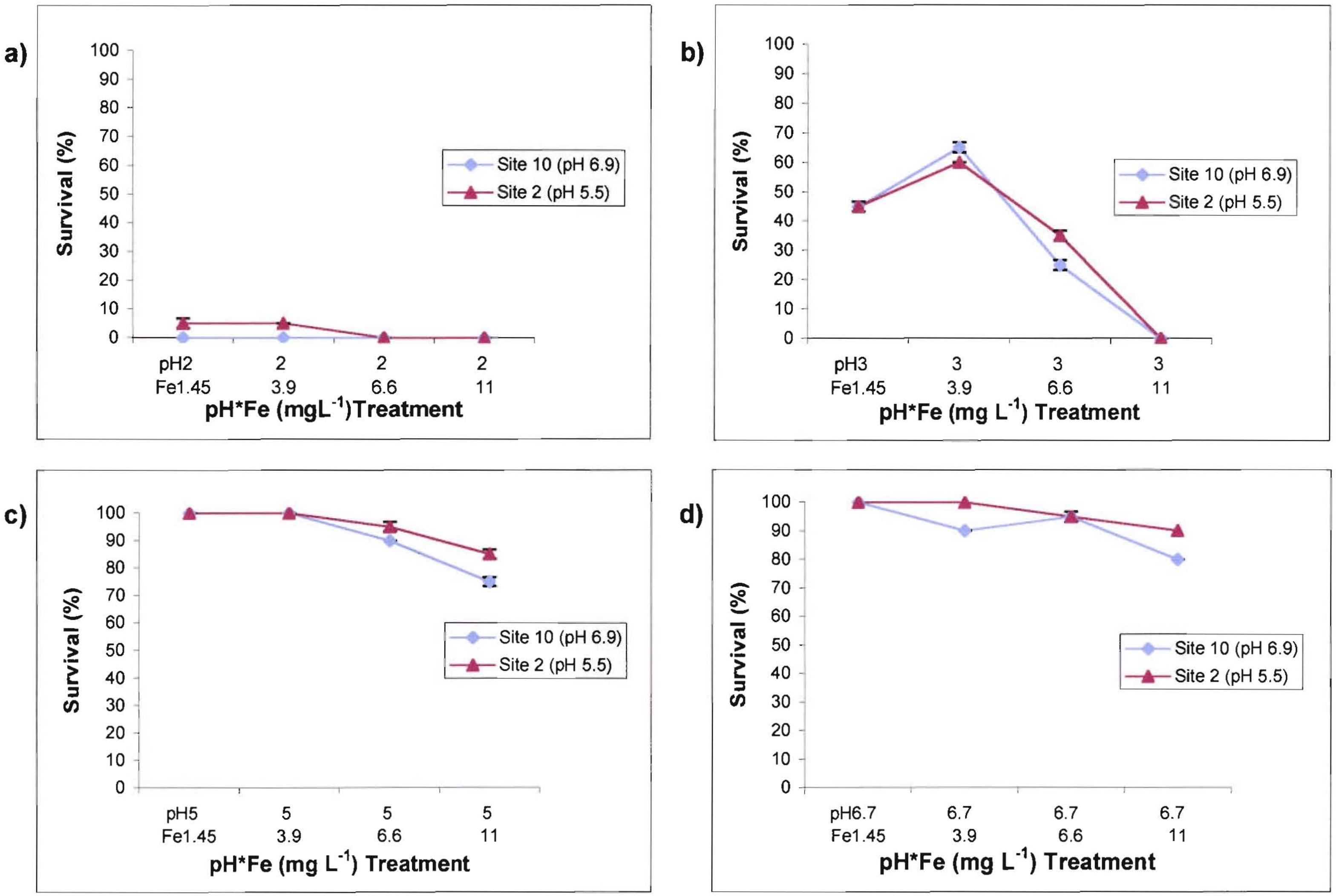


Fig 4.10 Percentage survival (± 1SE) of *Deleatidium larvae* collected from an acid site (site 2; pH 5.5) and a circumneutral site (Site 10; pH 6.9) and exposed to four iron concentrations (1.45, 3.9, 6.6 and 11 mg L^{-1}) at: **a)** pH 2 **b)** pH 3 **c)** pH 5 **d)** pH 6.7 (tap water control).

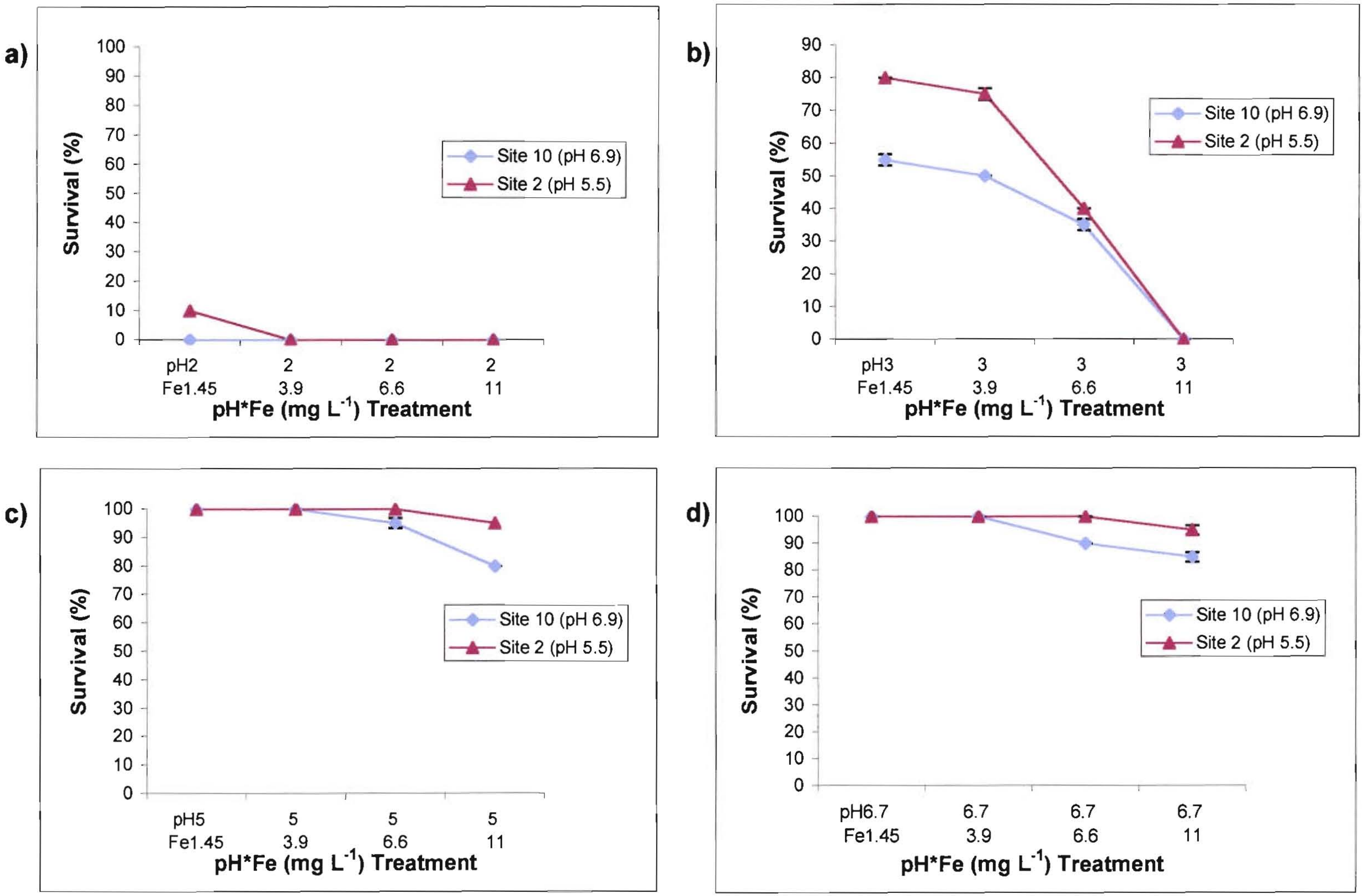


Fig 4.11 Percentage survival (± 1SE) of *Z.confusus* collected from an acid site (site 2; pH 5.5) and a circumneutral site (Site 10; pH 6.9) and exposed to four iron concentrations (1.45, 3.9, 6.6 and 11 mg L-1) at: **a)** pH 2 **b)** pH 3 **c)** pH 5 **d)** pH 6.7 (tap water control).

through habitat loss caused by the presence of an orange precipitate which blanketed the substrate and clogged the interstices. Hynes (1970) suggested that the presence of ferric hydroxide precipitate could eliminate many aquatic invertebrate species (Greenfield & Ireland, 1978; Scullion & Edwards, 1980; Gray, 1996).

The benthos of streams surveyed in this study comprised species that have been reported in other acid mine drainage-affected and circumneutral streams on the West Coast of the South Island (Winterbourn & McDiffett, 1996; Winterbourn, 1998). Winterbourn & McDiffett (1996) collected 16 taxa from sites contaminated by acid mine drainage on the Stockton-Denniston Plateau, and in the present study the total number collected from acid streams ranged from 14 – 20. Winterbourn (1998) reported lower abundances (1 – 10 taxa) in streams of pH 2.6 – 4.2 associated with the Buller and Reefton coalfields. Greenfield & Ireland (1978) found similar results to Winterbourn (1998) in streams affected by coalmine spoil in the Burnley area of Lancashire.

In the present study, the Plecoptera was the best represented insect order in streams with pH > 4.5, followed by Trichoptera and Diptera. Streams with pH < 4.5 were numerically dominated by Diptera, mainly Chironomidae, which have been reported to be very tolerant of acidic conditions elsewhere (Koryak, Sharpiro & Sykora, 1972; Greenfield & Ireland, 1978; Letterman & Mitsch, 1978; Winterbourn & Ryan, 1994; Winterbourn & McDiffett, 1996; Winterbourn, 1998). However, Winterbourn & McDiffett (1996) found that Diptera were numerically dominant in both circumneutral and acidic streams on the Stockton-Denniston Plateau in contrast to my findings. In the Northern Hemisphere, several authors (Sutcliffe & Carrick, 1973; Greenfield & Ireland, 1978; Feldman & Connor, 1992) have noted an absence of Ephemeroptera from streams with pH 5.4 – 5.7, and other studies (Letterman. & Mitsch, 1978; Scullion & Edwards, 1980) have reported a general absence of crustaceans from acidic environments. In my study, crustaceans were present at nine of the ten stream sites with the ostracod, *Herpetocypris pascheri* at pH 3.4, the amphipod, *Paraleptamphopus subterraneus* at pH 3, and harpacticoid copepods in water with pH as low as 2.9.

Generally, circumneutral streams are characterised by a large number of species and individuals, whereas streams exposed to acid effluents have a relatively smaller number of species and individuals present (Dills & Rogers, 1974; Scullion & Edwards, 1980; Hall, Driscoll & Likens, 1987; Mason, 1996; Winterbourn & McDiffett, 1996). My control sites had greater species richness and abundance than acidic streams in accordance with this generalisation and streams with pH > 4.5 had a more diverse range of taxa and abundances than sites with pH < 4.5. These findings are consistent with those of Winterbourn &

McDiffett (1996) and Winterbourn (1998) who noted that a small number of aquatic insects characteristic of "clean", stony streams occur down to a pH of about 4.

In the present study, one acid stream (Site 1), while having relatively low taxon diversity, also had one species, the stonefly, *Spaniocercoides philpotti* in high abundance. Similar situations have been reported by Koryak, Sharpiro & Sykora (1972) for streams receiving high levels of acid mine drainage in western Pennsylvania, where low species diversity (even as low as a single species) was associated with high numbers of individuals. Hildrew *et al.* (1984) also reported a similar phenomenon for some low pH streams in southern England (not mine drainages) where detritivorous stonefly densities were high even though total species numbers were low. They suggested that density compensation was occurring whereby reduced competition increased niche width and allowed generalist stonefly species to attain high densities.

Oligochaeta, the stoneflies *Cristaperla fimbria* and *Spaniocerca zelandica*, a caddisfly *Psilochorema* sp. (Hydrobiosidae), and dipterans in the families Ceratopogonidae, Chironomidae and Tipulidae, and Acarina were found at all sites surveyed. Furthermore, the mayfly, *Deleatidium* (Leptophlebiidae), two stoneflies *Nesoperla fulvescens* and *Spaniocercoides philpotti*, and caddisflies *Hydrobiosis* sp. and *Triplectides obsoleta* were found at many sites. Leptophlebiid mayflies and hydrobiosids (Winterbourn, 1981), and many species of stoneflies (Hildrew *et al.*, 1984) are considered to be moderately or very tolerant to changes in water quality, while three other caddisfly larvae have also been found in highly acidic West Coast streams (Winterbourn, 1998).

In the field *Deleatidium* tolerated a wide range of pH (3 – 7.1), Fe (0 – 3.5 mg L^{-1}) and Al (0 – 6.9 mg L^{-1}) conditions, and larvae were also found by Winterbourn (1998) at pH 3.5. The distributions of other mayflies, *Coloburiscus humeralis* (pH 6.8 – 7.1) and *Nesameletus* (pH 4.5 – 7.1) were more restricted with respect to pH than that of *Deleatidium*, although an earlier study of acid brown water streams in Westland found *Coloburiscus humeralis* down to pH 4.6 (Collier, 1988). Another mayfly, *Zephlebia*, was found at only one circumneutral site in my study, whereas Winterbourn & McDiffett (1996) found it at 12 sites on the Stockton-Denniston Plateau in the pH range 2.9 –6.1. The most widespread stonefly *Spaniocerca zelandica* was found at pH 3, while *Spaniocercoides philpotti* has a pH minimum of 2.9 which extends the pH range at which it has been reported. As found in earlier studies, *Psilochorema* was widely distributed in acid waters and its known pH minimum was also extended to pH 2.9.

Dills and Rogers (1974) reported little seasonal variation in the diversity of the invertebrate fauna of streams affected by acid mine drainage in North America, and no significant seasonal changes were observed in my study either with respect to invertebrate abundances at all ten sites combined. However, the stonefly, *S.philpotti* did exhibit seasonal changes and was absent from all streams in March.

As well as affecting surface waters, I found that acid mine drainage contaminated hyporheic waters, whose chemistry largely mirrored that of the surface water. Water conditions in the hyporheic zone were marginally less polluted than corresponding surface waters, however pH was still below 4 at five of the sites. The number of taxa collected from the hyporheic zone ranged from 5 at acidic sites to 16 at sites with pH > 4.5, whereas surface sediment samples had 14 to 40 taxa. Crustacea (mainly harpacticoid copepods), followed by Diptera (mainly Chironomidae) and Plecoptera numerically dominated all hyporheic sites and the strong representation of copepods was the distinguishing feature of their faunas. Nelson *et al.* (1993) collected 21 taxa from circumneutral sites and 11 taxa from acidic sites in hyporheic samples from the upper Arkansas River in central Colorado, and noted that the hyporheos of the acid mine-drainage affected site was dominated by Chironomid and other Diptera whereas surface samples were dominated by non-chironomid Diptera and Ephemeroptera.

The size and shape of the organisms may be a factor affecting distribution within the hyporheic zone. Slender animals with flexible bodies such as Chironomidae can probably move more easily between the substrate particles, while the extreme smallness of copepods, may enable them to swim in the interstitial water.

Several studies of the hyporheic zone in streams unaffected by acid mine drainage have shown Chironomidae and other Diptera, Acarina (McElravy & Resh, 1991; Boulton, Valett & Fisher, 1992; Marchant, 1995) and harpacticoid copepods and cyclopoids to be the dominant invertebrates (Boulton, Valett & Fisher, 1992; Marchant, 1995). Adkins (1997) reported that Chironomidae and some species of Trichoptera were dominant in streams in the Cass Basin, New Zealand (unaffected by acid mine drainage). Chironomidae were also the dominant hyporheic invertebrates in circumneutral Middle Bush Stream, along with an ostracod, *Darwinula* sp. (McLeod, 1998). Chironomidae were also present in the hyporheic zone in relatively high abundance in my study, both at sites with pH > and < 4.5, and although few ostracods were found in my study, the harpacticoid copepods were numerically dominant at sites affected and unaffected by acid mine drainage.

The lower abundances of invertebrates at acidic sites (pH < 4.5) than sites with pH > 4.5 could in part be due to the fact that the streambeds of acidic sites were often coated in a layer of fine-grained sediments such as mud and silt (described in Chapter 2), that also filled interstitial spaces and cannot be colonised by many animal groups (Bourassa & Morin, 1995). Similarly, the reduced abundances of invertebrates at Sites 4 and 5 (pH > 4.5 and control site, respectively) could have been due to the presence of sand on the steam bed. Sand provides a poor substrate for most macroinvertebrates because of its instability, tight packing of grains, and consequent paucity of interstitial spaces that limit water, nutrient and oxygen transport.

Finally, the lack of seasonal variation in abundance of the hyporheos at all ten sites contrasted with the finding of Adkins (1997) who reported peak densities of invertebrates in inland Canterbury streams in autumn when many new generation larvae are known to be present (Winterbourn, 1978, 1995; Winterbourn & Harding, 1993). McElravy & Resh (1991) also noted a period of increased abundance in the hyporheic zone in autumn following periods of oviposition, in Big Canyon Creek, California.

As well as demonstrating a wide range of sensitivity to acidity in the field, invertebrates have also been found to respond differently under laboratory conditions (Okland & Okland, 1986). Intraspecific variability in pH tolerance has been demonstrated for a number of species, including mayflies (Rowe *et al.*, 1989), stoneflies (Havas & Hutchinson, 1982) and amphipods (France & Stokes, 1987). In my study, tolerance experiments showed that *Deleatidium* and *Zelandobius confusus* larvae from moderately acidic streams survived better at pH 3 than animals from circumneutral streams. All animals died at pH 2.

Interspecific variability in tolerance of aquatic animals to low pH can also be considerable. Caddis species have been reported to be generally very tolerant of low pH, and *Brachycentrus americanus* even survived at pH 1.5 (Bell & Nebeker, 1969). I found that *Pycnocentrella eruensis* was more tolerant of low pH than the two mayfly and stonefly species, although this was not reflected in their field distributions. Some larvae of *Deleatidium* and *Zelandobius confusus* survived at pH 2 so it would be interesting to test these animals in even more acidic water.

Lastly, mortality of both *Deleatidium* and *Zelandobius confusus* larvae was low at all iron concentrations tested at pH 5 and 6.7, but increased rapidly at pH 3. Both species and *Pycnocentrella eruensis* had increased pH and iron tolerance in the lab than would have been predicted from field surveys. In the field, the mayfly were not present below pH < 3 and Fe > 3.5 mg L^{-1}, the stonefly, pH < 3 and Fe > 3.22 mg L^{-1} and the caddisfly, pH < 5.5 and Fe > 0.08 mg L^{-1}. Havas & Hutchinson (1982) noted that the addition of free iron to control pond water to achieve [Fe] 30 mg L^{-1} resulted in the formation of iron hydroxide and a drop in pH from 4.5 to 4.2 within 50 hours. Such a reduction in pH alone may have been sufficient to account for some of the increased mortality in my experiments although, after 96 hours the pH of test solutions was still within 10 % of its initial value. The iron tolerance experiments indicated that survival was poorer at a given pH when iron concentration was elevated above 6.6 mg L^{-1}, but at most of my field sites Fe concentrations were < 1.5 mg L^{-1} and its role in determining the distributions of stream invertebrates was probably minor.

Chapter Five

CONCLUDING DISCUSSION

The aim of my study was to investigate the effects of acid mine drainage on the physico-chemical conditions of surface and hyporheic receiving waters and to examine the relationship between these conditions and invertebrate community structure, and epilithic algal production. To achieve this, stream water chemistry, surface and hyporheic faunas and algal production of 10 streams associated with the Murray Creek Goldfield and Alborns Coal Mine near Reefton, South Island, were sampled over a 12 month period. Although research into the effects of acid mine drainage on invertebrate community structure in surface sediments has been studied in many countries, the effects on hyporheic fauna have received little attention, in spite of the reported importance of this zone to in-stream processes and invertebrate ecology. This study is the first to consider the effects of acid mine drainage on hyporheic fauna of New Zealand streams.

Natural streams exhibit wide variations in water chemistry as a result of differences in the geology, vegetation, soils and run-off characteristics of their catchment (Stewart, 1993). s Results of my water chemistry analysis of surface waters conformed with the findings of Dills & Rogers (1974), Letterman & Mitsch (1978), Winterbourn & McDiffett (1996) and Winterbourn (1998) that acid mine drainage affected streams are characterised by low pH, little or no measurable alkalinity and high conductivity, reflecting high concentrations of metal ions. Increased total dissolved aluminium and total iron were observed in the Reefton streams as pH declined. Several studies carried out on other streams affected by acid mine drainage, both in New Zealand (e.g., Winterbourn & McDiffett, 1996; Winterbourn, 1998) and overseas (e.g., Dills & Rogers, 1974; Scullion & Edwards, 1980; Raumussen & Lindgaard, 1988) have found strong relationships between the chemical nature of stream water and distributions of benthic invertebrates. Aquatic invertebrate species vary greatly in their tolerance of acid water. For example, a pH of < 6 combined with Fe > 3.6 mg L^{-1} reduced the number of invertebrate species in streams of the Ben's Creek Watershed, Pennsylvania to a quarter of the number present at sites with pH > 6 and Fe < 0.2 mg L^{-1} (Letterman & Mitsch, 1978).

In the Northern Hemisphere, reductions in species richness have been reported at about pH 5.7 in streams affected by acid precipitation (Sutcliffe & Hildrew, 1989) and acid mine drainage (Greenfield & Ireland, 1978; Feldman & Connor, 1992). Below this threshold, mayflies, some caddisflies, crustaceans and molluscs tend to disappear or become scarce. In contrast, a small number of studies of New Zealand West Coast streams affected by acid mine drainage reported low diversities of aquatic invertebrates (including species of Ephemeroptera, Trichoptera, Diptera and Plecoptera) characteristic of "clean", stony streams down to a pH of about 4 or even lower (Winterbourn & McDiffett, 1996; Winterbourn, 1998).

The results of my study are consistent with these other New Zealand findings, as streams with pH > 4.5 had similar species richness and densities of invertebrates as circumneutral pH sites. Below a pH of about 3.7, where concentrations of Fe and Al were > 0.8 mg L^{-1} and > 0.4 mg L^{-1} respectively, species richness and abundance decreased rapidly and faunas were dominated numerically by Diptera, mainly Chironomidae. Species of Chironomidae have been reported to be very tolerant of acidic conditions elsewhere (Greenland & Ireland, 1978; Letterman & Mitsch, 1978; Winterbourn & Ryan, 1994; Winterbourn, 1998), consistent with my findings. The Plecoptera was the best represented insect order, in terms of species and individuals, in streams with pH > 4.5, followed by Trichoptera and Diptera. Other studies have noted a general absence of crustaceans from acidic environments (Letterman & Mitsch, 1978; Scullion & Edwards, 1980), however, crustaceans were present at nine of the ten Reefton sites, including those affected by acid mine drainage and with pH < 4.

The ability of aquatic organisms to cope with stresses imposed by low pH and associated changes in water chemistry varies considerably (Collier *et al.*, 1990). This ability may be underestimated by studies based on field distributions rather than laboratory trials because other environmental factors (e.g., oxygen consumption, substrate type, food availability, increased competition and predation) may also affect the distribution and abundance of some invertebrates in acidic streams. Results of my laboratory experiments indicate that *Pycnocentrella eruensis* is more tolerant of low pH and elevated iron concentrations than the mayfly *Deleatidium* or the stonefly *Zelandobius confusus*, although this was not reflected in their field distributions. Furthermore, all three species had higher iron concentration thresholds and lower pH thresholds in the lab than would be predicted from field surveys.

The greater species richness of streams with pH > 4.5 reflects the greater diversity of benthic habitats and food resources available, in addition to their more equable water chemistry. In contrast, streams contaminated by acid mine drainage were often characterised by the presence of iron precipitates and heavy deposits of silt and mud (as discussed in Chapter 2) resulting in both habitat loss (through blanketing of the substrate and the clogging of interstices) and a decrease in available food for invertebrates. Epilithic algal biomass was particularly low at the most acidic sites (pH < 4.5) where metal concentrations were high and precipitates prevented the attachment of algae to solid surfaces and may have inhibited photosynthesis. Invertebrate species diversity and abundance at highly acidic sites is thus affected by smothering of substrata and by loss of their algal food source.

Water chemistry of hyporheic samples was very similar to that of surface water, at all sites, although conductivity and alkalinity tended to be marginally lower and pH marginally higher. In addition, to water chemistry being similar, the faunas of surface and hyporheic substrata had much in common. However, diversity of the hyporheos was reduced in comparison with corresponding surface sites, and Crustacea, followed by Diptera and Plecoptera dominated all hyporheic sites. The strong representation of harpacticoid copepods in the hyporheic zone was a notable and distinguishing feature of their faunas.

The findings of the present study indicate that not only are many New Zealand stream invertebrates found in both surface and hyporheic sediments, but at least on the West Coast of the South Island, they are also tolerant of low pH and moderately high concentrations of iron and aluminium. These findings are consistent with the prediction of Campbell & Stokes (1985) that aquatic organisms that can tolerate low pH should also tolerate elevated concentrations of metals because of the strong correlation between pH and metal concentrations in stream waters.

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Special thanks to Dad for his willingness and enthusiasm to come on every trip to Reefton and never complain when I made him carry all the heavy gear, climb up cliffs to try and find yet another stream for me to sample or wade almost knee deep into stagnant water for the "perfect photo". Your enthusiasm motivated me to keep plodding on many times when I just wanted to go back to the car and drive home.

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Appendix I

Stream channel stability evaluation form (after Pfankuch, 1975).

Item Rated	Stability Indicators by Classes							
UPPER BANKS	EXCELLENT		GOOD		FAIR		POOR	
Landform slope	Bank slope gradient <30%	(20)	Bank slope gradient 30-40%	(4)	Bank slope gradient 40-60%	(6)	Bank slope gradient 60%+	(5)
Mass-wasting (existing or potential)	No evidence of past or any potential for future mass-wasting into channel.	(3)	Infrequent and/or very small. Mostly healed over. Low future potential.	(6)	Moderate frequency and size, with some raw spots eroded by water during high flows.	(9)	Frequent or large, causing sediment nearly year-long OR imminent danger of same.	(12)
Debris jam potential (floatable objects)	Essentially absent from immediate channel area.	(2)	Present but mostly small twigs and limbs.	(4)	Present, volume and size are both increasing.	(6)	Moderate to heavy amounts, predominantly larger sizes.	(8)
Vegetative bank protection	90%+ plant density. Vigor and variety suggests a deep, dense, soil binding root mass.	(3)	70-90% density. Fewer plant species or lower vigor suggests a less dense or deep root mass.	(6)	50-70% density. Lower vigor and still fewer species form a somewhat shallow and discontinuous root mass.	(9)	<50% density plus fewer species and less vigor indicate poor, discontinuous and shallow root mass.	(12)
Channel capacity	Ample for present plus some increases. Peak flows contained. W/D ratio <7.	(1)	Adequate. Overbank flows rare. Width to Depth (W/D) ratio 8 to 15.	(2)	Barely contains present peaks. Occasional overbank floods. W/D ratio 15 to 25.	(3)	Inadequate. Overbank flows common. W/D ratio >25.	(4)
LOWER BANKS								
Bank rock content	65%+ with large, angular boulders 12"+ numerous.	(2)	40 to 65%, mostly small boulders to cobbles 6-12".	(4)	20 to 40%, with most in the 3-6" diameter class.	(6)	<20% rock fragments of gravel sizes, 1-3" or less.	(8)
Obstructions Flow deflectors Sediment traps	Rocks and old logs firmly embedded. Flow pattern without cutting or deposition. Pools and riffles stable.	(2)	Some present, causing erosive cross currents and minor pool filling. Obstructions and deflectors newer and less firm.	(4)	Moderately frequent, moderately unstable obstructions and deflectors move with high water causing bank cutting and filling of pools.	(6)'	Frequent obstructions and deflectors cause bank erosion year-long. Sediment traps full, channel migration occurring.	(8)
Cutting	Little or none evident. Infrequent raw banks less than 6" high generally.	(4)	Some, intermittently at outcurves and constrictions. Raw banks may be up to 12".	(6)	Significant. Cuts 12-24" high. Root mat overhangs and sloughing evident.	(12)	Almost continuous cuts, some over 24" high. Failure of overhangs frequent.	(16)
Deposition	Little or no enlargement of channel or point bars.	(4)	Some new increase in bar formation, mostly from coarse gravels.	(8)	Moderate deposition of new gravel and coarse sand on old and some new bars.	(12)	Extensive deposits of predominantly fine particles. Accelerated bar development.	(16
BOTTOM								
Rock angularity	Sharp edges and corners, plane surfaces roughened.	(1)	Rounded corners and edges, surfaces smooth and flat.	(2)	Corners and edges well rounded in two dimensions.	(3)	Well rounded in all dimensions, surfaces smooth.	(4)
Brightness	Surfaces dull, darkened or stained. Gen. not "bright".	(1)	Mostly dull, but may have up to 35% bright surfaces.	(2)	Mixture, 50-50% dull and bright, ±15%, i.e. 35-65%.	(3)	Predominantly bright, 65%+, exposed or scoured surfaces.	(4)
Consolidation or particle packing	Assorted sizes tightly packed and/or overlapping.	(2)	Moderately packed with some overlapping.	(4)	Mostly a loose assortment with no apparent overlap.	(6)	No packing evident. Loose assortment, easily moved.	(8)
Bottom size distribution and percent stable materials	No change in sizes evident. Stable materials 80-100%.	(4)	Distribution shift slight. Stable materials 50-80%.	(8)	Moderate change in sizes. Stable materials 20-50%.	(12)	Marked distribution change. Stable materials 0-20%.	(16
Scouring and deposition	Less than 5% of the bottom affected by scouring and deposition.	(6)	5-30% affected. Scour at constrictions and where grades steepen. Some deposition in pools.	(12)	30-50% affected. Deposits and scour at obstructions, constrictions, and bends. Some filling of pools.	(18)	More than 50% of the bottom in a state of flux or change nearly year-long.	(24
Clinging aquatic vegetation (moss and algae)	Abundant. Growth largely moss-like, dark green, perennial. In swift water too.	(1)	Common. Algal forms in low velocity and pool areas. Moss here too and swifter waters.	(2)	Present but spotty, mostly in backwater areas. Seasonal blooms make rocks slick.	(3)	Perennial types scarce or absent. Yellow-green, short term bloom may be present.	(4)
	COLUMN TOTALS							

Add the values in each column for a total reach score here (E___ + G___ + F___ + P___ = ___).

Reach score of: <38 = Excellent, 39-76 = Good, 77-114 = Fair, 115+ = Poor.

Appendix II
SURFACE SAMPLES

March

	Site														
	1A	1B	1C	2A	2B	2C	3A	3B	3C	4A	4B	4C	5A	5B	5C
ANNELIDA															
Oligochaeta indet.							5	3	6				2	6	4
PLATHELMINTHES															
Neppia montana													2	1	
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterraneus				1		3	2		1						
Copepoda															
Harpacticoid															
Ostracoda															
Herpetocypris pascheri															
INSECTA															
Ephemeroptera															
Austroclima sepia					1	1									2
Coloburiscus humeralis															
Deleatidium spp.	5	1	7	9	17	12	1	4	2	4	10	7	43	56	85
Neozephlebia scita				1											
Nesameletus sp.				2	3	4							3	4	9
Plecoptera															
Austroperla cyrene				2						5	2	6	3	2	7
Cristaperla fimbria				3	5	1	2		3		3	2	28	24	33
Megaleptoperla grandis				1	1										2
Nesoperla fulvescens	2			2	4		6	1	3			2	3	5	7
Spaniocerca zelandica	4	1	4	15	11	4	10	4	6				38	30	68
Stenoperla prasina perla			1			2				1	1	1	3	2	1
Zelandobius confu				3		1	2		1	5	3	9	5	1	4
Zelandobius fenes														1	1
Trichoptera															
Hydobiosella stenocerca	2	1													
Hydrobiosis sp.		1	2	3			2						2		1
Philorheithrus agilis											1	3		1	
Psilochorema sp.			1	1		2								1	
Pycnocentrella eruensis															
Triplectides obsoleta				1	1	3		1							
Coleoptera															
Hydraenidae	1		3	1	3		2	1	1		1	3	2	4	2
Hydrophilidae							1				1	3			
Ptilodactylidae															
Scirtidae															2
Diptera															
Ceratopogonidae				1	1										
Chironomidae	17	35	29	31	11	1	31	8	16	11	18	55	16	30	63
Eriopterini										4	2		3	6	4
Limonidae		1						1							
Muscidae	1	3								2	1				
Nothodixa sp.										1		2			
Paralimnophila skusei								1							1

Neuroptera															
Kempynus sp.								1							
Acarina				1			2		1						
TOTAL INVERTEBRATES	32	43	47	78	58	34	66	25	40	33	43	93	153	174	296
TOTAL TAXA	7	6	8	17	11	11	12	10	10	8	11	11	14	16	18

Appendix II
SURFACE SAMPLES

March continued	Site														
	6A	6B	6C	7A	7B	7C	8A	8B	8C	9A	9B	9C	10A	10B	10C
ANNELIDA															
Oligochaeta indet.	2	3	1	1	4	1	3	5	2	4	2	1	2		1
PLATHELMINTHES															
Neppia montana													2		1
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterraneus		1									2	1		1	
Copepoda															
Harpacticoid													3	5	7
Ostracoda															
Herpetocypris pascheri														1	
INSECTA															
Ephemeroptera															
Austroclima sepia													3		
Coloburiscus humeralis													1	6	6
Deleatidium spp.													63	101	89
Neozephlebia scita															
Nesameletus sp.													3		
Plecoptera															
Austroperla cyrene							2	1	1				2	3	2
Cristaperla fimbria													1	3	6
Megaleptoperla grandis															
Nesoperla fulvescens	1	4	3							1	3	1			
Spaniocerca zelandica	3	8	6							3	4	1	3	11	15
Stenoperla prasina													2	3	1
Zelandobius confu							2	2	3				6	19	15
Zelandobius fenes															
Trichoptera															
Hydobiosella stenocerca														2	1
Hydrobiosis sp.		3	1							1	1			5	2
Philorheithrus agilis															
Psilochorema sp.	3	5	2	2	1	1	4	4	3	2	1		3		1
Pycnocentrella eruensis														4	2
Triplectides obsoleta		1								1					
Coleoptera															
Hydraenidae													2	3	8
Hydrophilidae				1		1									
Ptilodactylidae													1	1	
Scirtidae															
Diptera															
Ceratopogonidae	1	1													
Chironomidae	13	34	18	3	2	7	11	8	18	7	3	7	43	52	31
Eriopterini	1	1												1	
Limonidae				2	3	5									
Muscidae		1	2								2	1			
Nothodixa sp.															1
Paralimnophila skusei			1												

Neuroptera															
Kempynus sp.															
Acarina							1		2				1	1	
TOTAL INVERTEBRATES	24	62	34	9	10	15	23	20	29	20	18	12	141	222	189
TOTAL TAXA	7	11	8	5	4	5	6	5	6	8	8	6	17	18	17

Appendix II
SURFACE SAMPLES

June	Site																								
	1A	1B	1C	1D	1E	2A	2B	2C	2D	2E	3A	3B	3C	3D	3E	4A	4B	4C	4D	4E	5A	5B	5C	5D	5E
ANNELIDA																									
Oligochaeta indet.						7	11			2	1	3	4	1	2		1			2	2	1		2	2
PLATHELMINTHES																									
Neppia montana						1										1			1			1	3		1
CRUSTACEA																									
Amphipoda																									
Paraleptamphopus subterraneus							2			3	2		1		8			1	1						
Copepoda																									
Harpacticoid						2	6	8	7	24	6	8		2	4	7	14	11	8	6		1			1
Ostracoda																									
Herpetocypris pascheri																					10	3		8	3
INSECTA																									
Ephemeroptera																									
Austroclima sepia																									
Coloburiscus humeralis																									
Deleatidium spp.			2	5	3	8	5	14	12	1	2	9	1	7		36	19	21	21	15	42	39	41	55	40
Neozephlebia scita																									
Nesameletus sp.									1	1						1	1	4							1
Zephlebia sp.																									
Plecoptera																									
Acroperla trivacuata																2		2			3		1	3	
Austroperla cyrene																9	4	3	1	2	5	2	2	3	1
Cristaperla fimbria			1		2		11	5	8	6	3	5	2		3	10		8	1		32	16	21	13	28
Megaleptoperla grandis							5			1	1													2	1
Nesoperla fulvescens					1						1			2		1			1						
Spaniocerca zelandica			3			2	4	5	6	1	2	3		1	2	2		4	3		10			5	4
Spaniocercoides philpotti	128	400	103	59	130						3	6	1	3	1			5	6	1	8	3			

Stenoperla prasina							3				1	1		2	1	2	1	5	2	2				2	
Zelandobius confusus						2	1			1						12	6	25	11	16		3	5	1	1
Zelandobius fenestrata						1										1									
Trichoptera																									
Aoteapsyche sp.																					2				
Hydobiosella stenocerca																6	11	5	1		1	1		1	1
Hydrobiosis sp.					2	1	2	1		1			1	1											
Oxyethira albiceps													1		1										
Philorheithrus agilis							3						1			1	1	1							
Polyplectopus sp.										1	2					2					2			2	2
Psilochorema sp.	2	3	3	1	1						1												1	1	2
Pycnocentrella eruensis																									
Triplectides obsoleta										1	6		1		12										
Coleoptera																									
Hydraenidae					1					1											3		1		
Hydrophilidae							1										1		3	1	1			3	
Ptilodactylidae							1									1		1		2					
Scirtidae						1																		1	
Diptera																									
Austrosimulium sp.																22	8	2	3	7					
Ceratopogonidae							1	1	1																
Chironomidae	6	31	21	10	16	12	40	10	15	32	22	16	25	18	20	88	55	61	43	51	47	33	39	28	32
Eriopterini									1	1						2	3	4	2	1	5	2	1	6	15
Limonidae		1		1											1		1				2				
Nothodixa sp.																		1	2		4	1		3	
Paralimnophila skusei																									
Other Dipteran larvae		4															1								
Mecoptera																									
Nannochorista philpotti						1																			
Neuroptera																									
Kempynus sp.																									
Acarina	1	1	1		1			1		2	1	1		1	1	1	1	3	1	1	1	1	1	4	
TOTAL INVERTEBRATES	137	440	134	76	157	38	96	45	51	79	54	52	38	38	56	207	128	167	111	107	180	107	116	143	135
TOTAL TAXA	4	5	7	5	9	11	15	7	8	17	15	9	10	10	12	20	16	19	18	13	18	14	11	19	17

Appendix II
SURFACE SAMPLES

June continued

	6A	6B	6C	6D	6E	7A	7B	7C	7D	7E	8A	8B	8C	8D	8E	9A	9B	9C	9D	9E	10A	10B	10C	10D	10E
ANNELIDA																									
Oligochaeta indet.	1			3	1						1	1	3	1	2	3	1	2			6		1	5	
PLATHELMINTHES																									
Neppia montana																					1	2		1	
CRUSTACEA																									
Amphipoda																									
Paraleptamphopus subterraneus																					2				2
Copepoda																									
Harpacticoid	10	5	3	5	7	4	3		2				2		3	1					5	11	2	9	
Ostracoda																									
Herpetocypris pascheri																							1	3	
INSECTA																									
Ephemeroptera																									
Austroclima sepia																					2				7
Coloburiscus humeralis																					3	19	6	4	13
Deleatidium spp.	1																				111	150	80	91	153
Neozephlebia scita																									6
Nesameletus sp.																					2	1		3	8
Zephlebia sp.																						2			
Plecoptera																									
Acroperla trivacuata																									
Austroperla cyrene																					1	6	3	1	7
Cristaperla fimbria		1				5	2	2	5	1											11	1	5	1	23
Megaleptoperla grandis																									
Nesoperla fulvescens						2		1	3									2					3		3
Spaniocerca zelandica						11	3	5	6	4						2	2	5	2	4	5		8		28
Spaniocercoides philpotti		1																							

Stenoperla prasina																					1	3	4	1	3
Zelandobius confusus																					8	4	21	2	17
Zelandobius fenestrata																									
Trichoptera																									
Aoteapsyche sp.																									
Hydobiosella stenocerca																						1		1	
Hydrobiosis sp.	1	1					1		1								1	1			1	5			12
Oxyethira albiceps																									
Philorheithrus agilis																									1
Polyplectopus sp.																									
Psilochorema sp.			1	1	1	3	2	1	1												2	3	2	1	7
Pycnocentrella eruensis																						2	1	2	
Triplectides obsoleta									5											5					
Coleoptera																									
Hydraenidae																					8		6		12
Hydrophilidae																									
Ptilodactylidae																					1		1		2
Scirtidae																									
Diptera																									
Austrosimulium sp.																						5			
Ceratopogonidae	1			1		2	1		1				1					2		1					
Chironomidae	95	75	174	89	71	21	11	8	17	14	1	10	7	3	8	4	9	2	7	38	21	30	19	13	68
Eriopterini																						2			3
Limonidae			1					1	1		1		1					1							
Nothodixa sp.																					1	1			2
Paralimnophila skusei		2																							
Other Dipteran larvae		31	2	3	1	10		3	2	5		3	2	2	1	5		1							
Mecoptera																									
Nannochorista philpotti																									
Neuroptera																									
Kempynus sp.								1																	
Acarina	2	2		1	1	2				1												1	1	3	
TOTAL INVERTEBRATES	111	118	181	103	82	60	23	22	43	25	3	14	16	6	14	15	13	16	9	48	192	249	164	141	377
TOTAL TAXA	7	8	5	7	6	9	7	8	11	5	3	3	6	3	4	5	4	8	2	4	19	19	17	16	20

Appendix II
SURFACE SAMPLES

September

	1A	1B	1C	1D	1E	2A	2B	2C	2D	2E	3A	3B	3C	3D	3E	4A	4B	4C	4D	4E	5A	5B	5C	5D	5E
ANNELIDA																									
Oligochaeta indet.		1	1			4	1	4	2	3	7	2	11	7	4				1		1	6	2	2	9
PLATHELMINTHES																									
Neppia montana						1	2		1										1		11	7	13	4	7
CRUSTACEA																									
Amphipoda																									
Paraleptamphopus subterraneus													1	2							1			1	
Copepoda																									
Harpacticoid						2	12	3	3	14	9	3	6	25	6		10	1	34		1	2	3	8	4
Ostracoda																									
Herpetocypris pascheri							1		4	5				7	1						10	7	10	5	9
Decapoda																									
Paranephrops planifrons																									
INSECTA																									
Ephemeroptera																									
Coloburiscus humeralis																									
Deleatidium spp.		3	1			6	23	14	10	18		2				22	23	31	40	31	73	69	81	59	53
Neozephlebia scita																									
Nesameletus sp.								1	1	6						3		1							
Zephlebia sp.																									
Plecoptera																									
Austroperla cyrene						2	2	1	2	4						8	9	9	11	5		3		4	2
Cristaperla fimbria			2			19	24	8	4	33	5	2		3	4	22	9	15	12	10	18	11	21	3	3
Megaleptoperla grandis						1														1					
Nesoperla fulvescens						2	2																1		
Spaniocerca zelandica						3	1	3			2				2	8	2	8		16	4	2	4	6	
Spaniocercoides philpotti	119	101	206	130	108	1		2	1	1	2	11		2	16	6	1	7		3	1				1
Stenoperla prasina							1				1		1			4	5		3	2		1			
Taraperla sp.							55	26	17	25						4	8	8		6					
Zelandobius confusus						4	9	1	1	4					1	13	17	7	6	8	1	1	1		
Zelandobius fenestrata						8	3	8	1	1						4	7		6	9		3			

Trichoptera																									
Aoteapsyche sp.																						1			
Hydobiosella stenocerca						1	1									8	11	6	8						1
Hydrobiosis sp.							1	1						1			2	2	13		1	3	2		1
Philorheithrus agilis							7		1														1	1	1
Polyplectopus sp.									1					1					1						
Psilochorema sp.	8	6	4	4	3								1			1	2	2							
Pycnocentria sylvestris																					4	1	2		2
Triplectides obsoleta										32				8	5	3	4	3	3	2					
Zelandopsyche maclellani								1	1	2								2		1					
Coleoptera																									
Hydraenidae						1	1			2					1	9	4	18	6	20	10	2	8	5	3
Hydrophilidae						1																	1		
Ptilodactylidae						1											1				1				
Scirtidae						1										1		1		1					
Diptera																									
Austrosimulium sp.																4	6	4	8		4	1	1	2	1
Ceratopogonidae			1				1		3	3				4	2	3		2	4	3		1	2		
Chironomidae	1	1	1	4	15	68	78	22	7	69	4	12	17	12	54	102	149	108	300	116	50	33	58	20	31
Eriopterini																2			2	1	1	13	3	10	2
Limonidae							1		1						1				1		1			2	
Neocurupira tonnoiri																									
Nothodixa sp.																1	1			1					
Other Dipteran larvae						2				1		1	2	1											1
Paralimnophila skusei																	1		1						
Neuroptera																									
Kempynus sp.						1																			
Acarina	1				1		1			2	1						1	3		1					
TOTAL INVERTEBRATES	129	112	216	138	127	129	227	95	61	225	31	33	39	73	97	228	273	238	461	237	193	167	214	132	131
TOTAL TAXA	4	5	7	3	4	20	21	14	18	18	8	7	7	12	12	21	21	20	20	19	19	19	19	15	16

SURFACE SAMPLES

September continued

	6A	6B	6C	6D	6E	7A	7B	7C	7D	7E	8A	8B	8C	8D	8E	9A	9B	9C	9D	9E	10A	10B	10C	10D	10E
ANNELIDA																									
Oligochaeta indet.										1	2		3	1	1	1	5	4			6				
PLATHELMINTHES																									
Neppia montana																					1	1	1	1	1
CRUSTACEA																									
Amphipoda																									
Paraleptamphopus subterraneus																									
Copepoda																									
Harpacticoid		2				3	1		1		2	3	7	4	1			1	1		4	1			
Ostracoda																									
Herpetocypris pascheri			1																		1		8		5
Decapoda																									
Paranephrops planifrons																						1			
INSECTA																									
Ephemeroptera																									
Coloburiscus humeralis																					18	24	13	1	9
Deleatidium spp.					2						1			1	2						202	102	120	67	76
Neozephlebia scita																						3	1		
Nesameletus sp.																					4	3	2	2	6
Zephlebia sp.																						1	1	1	
Plecoptera																									
Austroperla cyrene																					2	2		3	
Cristaperla fimbria					1																				
Megaleptoperla grandis																									
Nesoperla fulvescens																									
Spaniocerca zelandica															1										
Spaniocercoides philpotti	1	1			3	108	66	6	38	6	1	1	2	3	1	12	1	2	7	9					
Stenoperla prasina																					2	5	4		
Taraperla sp.																									
Zelandobius confusus																						1	1		
Zelandobius fenestrata																					1		4		1

Trichoptera																									
Aoteapsyche sp.																						2			1
Hydobiosella stenocerca																					8	22	9	30	3
Hydrobiosis sp.	1	1	2							1											7	1			
Philorheithrus agilis																									
Polyplectopus sp.																									1
Psilochorema sp.		3			3		2															2	6	1	1
Pycnocentria sylvestris																									
Triplectides obsoleta						2	3		1	1											1			1	
Zelandopsyche maclellani																									
Coleoptera																									
Hydraenidae																					3	1	2	3	1
Hydrophilidae									1													1			
Ptilodactylidae																					6	1	1		1
Scirtidae							1	1																	
Diptera																									
Austrosimulium sp.																					5	7	4	2	11
Ceratopogonidae								1		2	2	2		2		2	5		1	5	5	1	3	1	
Chironomidae	35	135	78	182	65	13	16	17	16	15	5	10	7	23	3	40	28	6	30	5	13	16	15	11	10
Eriopterini		1		1																	1	5	1	1	
Limonidae	2		1					1	1	1		1											1		
Neocurupira tonnoiri																								1	4
Nothodixa sp.																						1			
Other Dipteran larvae	5	5				3	2	4	7		1	6	5	1				3							
Paralimnophila skusei				1													1				1		1		
Neuroptera																									
Kempynus sp.																									
Acarina			1		1	1					1					1		1			1				4
TOTAL INVERTEBRATES	44	148	83	184	75	130	91	30	65	27	15	23	24	35	9	56	40	17	39	19	292	204	198	126	135
TOTAL TAXA	5	7	5	3	6	6	7	6	7	7	8	5	5	7	6	5	5	6	4	3	21	22	20	15	16

Appendix II
SURFACE SAMPLES

January

	1A	1B	1C	1D	1E	2A	2B	2C	2D	2E	3A	3B	3C	3D	3E	4A	4B	4C	4D	4E	5A	5B	5C	5D	5E
ANNELIDA																									
Oligochaeta indet.						5	1	2		3	4	7	1	1	4	3	2		6	1	5	1		1	1
PLATHELMINTHES																									
Neppia montana																1	1	2		1					
CRUSTACEA																									
Amphipoda																									
Paraleptamphopus subterraneus															2		2				1		1		
Copepoda																									
Harpacticoid						15	5	6	10	3	3	1	6	2	6	4	7					1		3	1
Ostracoda																									
Herpetocypris pascheri																	1		2						
INSECTA																									
Ephemeroptera																									
Austroclima sepia																								1	
Coloburiscus humeralis																									
Deleatidium spp.						13	10	18	5	8	15	22	4	3	3	21	27	11	8	16	8	66	12	23	31
Neozephlebia scita																									
Nesameletus sp.						1	2		1							3	2		1	2	1			1	
Zephlebia sp.																									
Plecoptera																									
Austroperla cyrene																5	2	1	5		1	7	1		3
Cristaperla fimbria						3	17	8	11	4	6	3	2	3		5	3	7	1		5	27	11	5	18
Megaleptoperla grandis						1																			
Nesoperla fulvescens							1																		
Spaniocerca zelandica						3	9	3	1	5	1					4	1					9	3	1	1
Spaniocercoides philpotti														1	5	1									
Stenoperla prasina																1			2			3		2	
Taraperla sp.																						7			
Zelandobius confusus									6	3						4	2	1	1	2		6	1		2
Zelandobius fenestrata						1										5	1	5	3			2		1	
Trichoptera																									

Aoteapsyche sp.																					
Hudsonema sp.																					
Hydobiosella stenocerca												6	3		7	1	2	8	3	5	
Hydrobiosis sp.			2		1	1	1		3	2		5	2	1	2	2					
Philorheithrus agilis			1																		
Psilochorema sp.							1				1	4	2	1	1			2	1	1	1
Pycnocentrella eruensis													3								
Triplectides obsoleta							1				1			2			1	1		2	
Zelandopsyche maclellani																					
Coleoptera																					
Hydraenidae												2	4		1	2	1	11	1	5	9
Hydrophilidae												2		3	1						
Ptilodactylidae			2	1											2						
Scirtidae												1	1	1	2	2					
Diptera																					
Austrosimulium sp.												1						5	1	3	8
Ceratopogonidae												3									
Chironomidae		11	20	6	9	11	32	22	19	38	9	86	55	84	78	39	24	93	51	69	58
Eriopterini			1											1	3			1			
Limonidae									1	1	1		1				1				
Nothodixa sp.													3					1			1
Paralimnophila skusei																					
Other Dipteran larvae							1		1												
Neuroptera																					
Kempynus sp.												1				1					
Acarina			1		1	1	1	2	1		1	3	2				2	1	5		1
TOTAL INVERTEBRATES		53	72	44	45	39	66	57	38	51	33	171	127	120	126	69	52	252	91	123	135
TOTAL TAXA		9	13	7	9	9	11	6	9	8	10	23	22	13	18	11	12	19	12	15	13

Appendix II
SURFACE SAMPLES

January continued

	6A	6B	6C	6D	6E	7A	7B	7C	7D	7E	8A	8B	8C	8D	8E	9A	9B	9C	9D	9E	10A	10B	10C	10D	10E
ANNELIDA																									
Oligochaeta indet.	1	4	2	4	2		1				1		2		2	1					1	3			1
PLATHELMINTHES																									
Neppia montana																					2		1		
CRUSTACEA																									
Amphipoda																									
Paraleptamphopus subterraneus														1		1				1	2	1	1		
Copepoda																									
Harpacticoid	3	2	3	5	6				2												9	3	1	2	1
Ostracoda																									
Herpetocypris pascheri																						1		1	
INSECTA																									
Ephemeroptera																									
Austroclima sepia																					1				
Coloburiscus humeralis																					2	8	3	11	5
Deleatidium spp.	3	2	2	3																	111	93	64	78	89
Neozephlebia scita																							1		
Nesameletus sp.																					1	2	1		
Zephlebia sp.																									
Plecoptera																									
Austroperla cyrene																				1	1		2		1
Cristaperla fimbria	1		3	5	2	5		1	6	5	1	2	2				3		1	1			3	4	1
Megaleptoperla grandis																									
Nesoperla fulvescens									1							1									
Spaniocerca zelandica						2			3	3	4	5	2	1		2	1		1	1	3	9	1	4	1
Spaniocercoides philpotti	1		1	1	3	7	3	5	7	5															
Stenoperla prasina																					1	2	1	1	2
Taraperla sp.																									
Zelandobius confusus																					11	2	3	1	
Zelandobius fenestrata																									
Trichoptera																									

Aoteapsyche sp.																					1				
Hudsonema sp.										1															
Hydobiosella stenocerca																					9	3	5	1	
Hydrobiosis sp.									2	1												1		3	1
Philorheithrus agilis																									1
Psilochorema sp.	1						1	2	1										1			1	3	1	1
Pycnocentrella eruensis																					1	1	2	2	
Triplectides obsoleta																									
Zelandopsyche maclellani																									1
Coleoptera																									
Hydraenidae																					1	1	1	2	3
Hydrophilidae																						1	2	1	
Ptilodactylidae																					1	1	1	1	2
Scirtidae																									1
Diptera																									
Austrosimulium sp.																							1		1
Ceratopogonidae												2			1		1			1	1		2	1	
Chironomidae	37	25	73	48	55	13	8	5	10	20	19	7	22	10	7	7	7	2	16	4	11	15	12	20	31
Eriopterini																							1		
Limonidae							1	1		1							1								
Nothodixa sp.																									
Paralimnophila skusei	2					1				1															
Other Dipteran larvae	13	18	4	7	9	3	5	3	2	5	1	1	2	3	1	3	4	1	2	3					
Neuroptera																									
Kempynus sp.																									
Acarina	2	4			3			1	1	2		1		2						1	1	2	2	1	1
TOTAL INVERTEBRATES	64	55	88	73	80	31	19	18	35	44	26	18	30	17	11	15	17	3	21	13	171	150	114	135	144
TOTAL TAXA	10	6	7	7	7	6	6	6	11	10	5	5	6	4	5	6	6	2	5	8	20	19	23	18	18

Appendix III
HYPORHEIC SAMPLES

March	Site														
	1A	1B	1C	2A	2B	2C	3A	3B	3C	4A	4B	4C	5A	5B	5C
ANNELIDA															
Oligochaeta indet.	3	1	2	1	3		3	4	3	2		1	2		1
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterraneus		1		1	4	6	5	1	2	2	1	1	1	3	3
Copepoda															
Harpacticoid		3	1	10	17	13	18	15	10	5	4	2	4	7	10
Cyclopoid				2	2	4				1	1			2	2
Ostracoda															
Herpetocypris pascheri				1			3				2	4			
INSECTA															
Ephemeroptera															
Ameletopsis sp.					2		1								
Plecoptera															
Spaniocerca zelandica						2	2	1	5						
Stenoperla prasina					2										
Cristaperla fimbria	1		5	2	7	3				2		1			
Trichoptera															
Hudsonema sp.					2										
Diptera															
Chironomidae			1	5	5	1	4	2	3	1	4	1	1		1
Other Dipteran larvae					1										
Acarina	1	1	2	2	2	2	2				6			1	3
TOTAL INVERTEBRATES	5	6	11	24	47	31	38	23	24	13	18	10	8	13	20
TOTAL TAXA	3	4	5	8	10	7	8	5	6	6	6	6	4	4	6

Appendix III
HYPORHEIC SAMPLES

March continued

	Site														
	6A	6B	6C	7A	7B	7C	8A	8B	8C	9A	9B	9C	10A	10B	10C
ANNELIDA															
Oligochaeta indet.				3	5	1				3	2	5	6	9	8
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterraneus				1	4	2	1	2	2				8	5	3
Copepoda															
Harpacticoid	1	1	1	3	3	6	2	4	8	1		2	38	41	29
Cyclopoid									1				8	6	9
Ostracoda															
Herpetocypris pascheri													1	2	
INSECTA															
Ephemeroptera															
Ameletopsis sp.													2	1	
Plecoptera															
Spaniocerca zelandica										2	1		10	8	9
Stenoperla prasina													1	2	1
Cristaperla fimbria	2		1										9	5	8
Trichoptera															
Hudsonema sp.								2	1				1		2
Diptera															
Chironomidae		5	2	2	1				1	1	3	1	11	7	9
Other Dipteran larvae															
Acarina	1				1	1	1				1		3	2	2
TOTAL INVERTEBRATES	4	6	4	9	14	10	4	8	13	7	7	8	98	88	80
TOTAL TAXA	3	2	3	4	5	4	3	3	5	4	4	3	12	11	10

Appendix III
HYPORHEIC SAMPLES

June

	Site														
	1A	1B	1C	2A	2B	2C	3A	3B	3C	4A	4B	4C	5A	5B	5C
ANNELIDA															
Oligochaeta indet.					5	2	6	5	6	1	1	2	8	2	2
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterraneus	1	3						3	5				1		
Copepoda															
Harpacticoid	11	25	30	3	10	14	2	63	35	1	1		3	6	1
Cyclopoid		1													
Ostracoda															
Herpetocypris pascheri	1	1										1		1	1
INSECTA															
Ephemeroptera															
Ameletopsis sp.															
Deleatidium sp.				1											
Plecoptera															
Spaniocerca zelandica				1	2	5							3	2	
Stenoperla prasina															
Cristaperla fimbria	6	2		2		2	2	5					5		
Spaniocercoides philpotti	4	1													
Trichoptera															
Hudsonema sp.						1		1	3						
Diptera															
Chironomidae		2	1	1	4	8	3	2	1	3	1	3	4	6	1
Limonidae															
Other Dipteran larvae					2	3						2	2	1	
Acarina	2	1	3		2	1	1		1	3	1	1		1	
TOTAL INVERTEBRATES	24	36	34	8	25	36	14	79	51	8	4	9	26	19	5
TOTAL TAXA	6	8	3	5	6	8	5	6	6	4	4	5	7	7	4

Appendix III
HYPORHEIC SAMPLES

June continued

	Site														
	6A	6B	6C	7A	7B	7C	8A	8B	8C	9A	9B	9C	10A	10B	10C
ANNELIDA															
Oligochaeta indet.				3	3	4		3			1	2	18	12	9
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterraneus							1						18	7	2
Copepoda															
Harpacticoid	3	1	1	3	1	3	3	8	5	2	2	1	62	43	39
Cyclopoid													8	2	3
Ostracoda															
Herpetocypris pascheri															3
INSECTA															
Ephemeroptera															
Ameletopsis sp.													5	1	
Deleatidium sp.															
Plecoptera															
Spaniocerca zelandica				1							2		12	10	1
Stenoperla prasina													3		
Cristaperla fimbria													23	11	8
Spaniocercoides philpotti															
Trichoptera															
Hudsonema sp.	1					1							2		
Diptera															
Chironomidae	3	4	1		1	2	3		1	2	2	1	12	15	21
Limonidae				1	2										
Other Dipteran larvae		1	2	1	1	2							1		
Acarina			1			1		3					3	1	4
TOTAL INVERTEBRATES	7	6	5	9	8	13	7	14	6	4	7	4	167	102	90
TOTAL TAXA	3	3	4	5	5	6	3	3	2	2	4	3	12	9	9

Appendix III
HYPORHEIC SAMPLES

September	Site														
	1A	1B	1C	2A	2B	2C	3A	3B	3C	4A	4B	4C	5A	5B	5C
ANNELIDA															
Oligochaeta indet.	1		2	2	2	3	2	1	3	1		2	2	1	1
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterraneus				3	2		1	3	2	1	1		1	1	
Copepoda															
Harpacticoid	9	7	21	18	11	15	25	30	28	3	1	1	7	4	9
Cyclopoid			6	1		2				1	1			2	
Ostracoda															
Herpetocypris pascheri								1			1	2			1
INSECTA															
Ephemeroptera															
Deleatidium sp.				2	1										
Plecoptera															
Spaniocerca zelandica				1	3	2	2		1		1		2	1	3
Stenoperla prasina				1											
Cristaperla fimbria				3	2	2	1	3	1	2		1	1	1	
Trichoptera															
Hudsonema sp.															
Diptera															
Chironomidae		1		5	3	6	2	2	6	3	2	2	3	1	3
Other Dipteran larvae				1					1					2	
Acarina	1			1	2	1	1		2	1	3	1	1		1
TOTAL INVERTEBRATES	11	8	29	38	26	31	35	40	44	12	10	9	17	13	18
TOTAL TAXA	3	2	3	11	7	7	7	6	8	7	7	6	8	9	7

Appendix III
HYPORHEIC SAMPLES

September continued

	Site														
	6A	6B	6C	7A	7B	7C	8A	8B	8C	9A	9B	9C	10A	10B	10C
ANNELIDA															
Oligochaeta indet.	2	3			1	5	1	2	3	3		4		12	9
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterraneus														3	1
Copepoda															
Harpacticoid	4	1	2	9	2	8	3	4	7	3		1	13	26	15
Cyclopoid													3	3	
Ostracoda															
Herpetocypris pascheri													5	3	2
INSECTA															
Ephemeroptera															
Deleatidium sp.															
Plecoptera															
Spaniocerca zelandica						2									
Stenoperla prasina															
Cristaperla fimbria															
Trichoptera															
Hudsonema sp.						1									
Diptera															
Chironomidae			4	1		4			1		2	1		7	7
Other Dipteran larvae		1				1							2	1	6
Acarina		2						1			1	1		2	3
TOTAL INVERTEBRATES	6	7	6	10	3	21	4	7	11	6	3	7	23	57	43
TOTAL TAXA	2	4	2	2	2	6	2	3	3	2	2	4	4	8	7

Appendix III
HYPORHEIC SAMPLES

January	Site														
	1A	1B	1C	2A	2B	2C	3A	3B	3C	4A	4B	4C	5A	5B	5C
ANNELIDA															
Oligochaeta indet.		1		1	2	3	2	6	3	1		1	5	1	
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterranesus	1						2		1	2	1		1	2	1
Copepoda															
Harpacticoid	9	5	11	17	12	13	29	22	30	1	3	2	16	13	5
Cyclopoid				1	2	1					1		2		1
Ostracoda															
Herpectocypris pascheri															1
INSECTA															
Ephemeroptera															
Deleatidium sp.					1	2									
Plecoptera															
Spaniocerca zelandica					2	1								2	
Stenoperla prasina											1				
Cristaperla fimbria		2	1				3	1	1	1		2		1	1
Spaniocercoides philpotti	2	2		1	2										
Trichoptera															
Hudsonema sp.								1							
Diptera															
Chironomidae	2	1	4	5	11	8	8	5	3	1	4	3	2	1	3
Limonidae					1										
Other Dipteran larvae								1				1	1		
Acarina	1	1		1	1	2	1	2	3	1	2			1	1
TOTAL INVERTEBRATES	15	12	16	26	34	29	45	38	40	7	12	9	32	21	13
TOTAL TAXA	5	6	3	6	9	6	6	7	6	6	6	5	6	7	7

Appendix III
HYPORHEIC SAMPLES

January continued	Site														
	6A	6B	6C	7A	7B	7C	8A	8B	8C	9A	9B	9C	10A	10B	10C
ANNELIDA															
Oligochaeta indet.		3	1	2	1	1		2	1		3	2	7	5	8
CRUSTACEA															
Amphipoda															
Paraleptamphopus subterranesus				1				1		1		1	1	3	1
Copepoda															
Harpacticoid	2	3	2	4	6	1	3	3	4	6	2	3	33	21	29
Cyclopoid													1	4	2
Ostracoda															
Herpectocypris pascheri													1		
INSECTA															
Ephemeroptera															
Deleatidium sp.															
Plecoptera															
Spaniocerca zelandica									1				9	5	5
Stenoperla prasina													1		1
Cristaperla fimbria													1	2	4
Spaniocercoides philpotti															
Trichoptera															
Hudsonema sp.		1													
Diptera															
Chironomidae	2		3	2	1	2	3		1	1	2	2	3	5	6
Limonidae															
Other Dipteran larvae						1			1						1
Acarina	1		2	2				1		1			1	2	2
TOTAL INVERTEBRATES	5	7	8	11	9	6	6	7	8	9	7	8	58	47	59
TOTAL TAXA	3	3	4	5	4	5	2	4	5	4	3	4	10	8	10